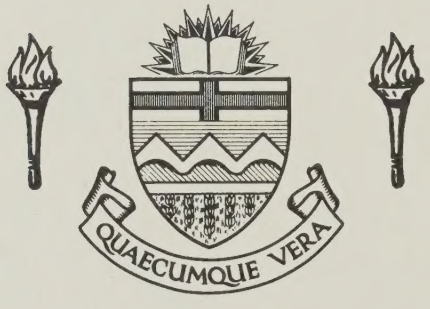

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THE UNIVERSITY OF ALBERTA

STRUCTURE-ACTIVITY STUDIES OF HYPOTENSIVE AGENTS

by

(C) MICHAEL LIONEL SELLEY

A THESIS

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TO ANGELA

ABSTRACT

In the light of knowledge gained by a previous group of investigators, an extended series of 3-monoalkylaminopropionohydroxamic acids were synthesized and examined for their hypotensive activities using anaesthetized rats. The results are discussed in terms of those structural features contributing to activity in these compounds.

The 3-monoalkylaminopropionohydroxamic acids were screened for their effectiveness in protecting mice against poisoning by di-isopropyl-fluorophosphonate (DFP). None of these compounds was found to be effective in protecting mice against organophosphate poisoning.

The 3-monoalkylaminopropionohydroxamic acids were all obtained by the interaction of the appropriate primary amine with methyl acrylate or methyl methacrylate and subsequent treatment of the methyl 3-monoalkylaminopropionates with hydroxylamine hydrochloride.

Several of the methyl 3-monoalkylaminopropionate precursors were also evaluated pharmacologically. The hypotensive activity of one of these compounds, namely 2-methyl-3-octylaminopropionate (103) was found to be the result of ganglionic blockade.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

The following abbreviations have been used throughout the main body of the text:

ACh	Acetylcholine
AChE	Acetylcholinesterase
DFP	Di-isopropylfluorophosphonate
Ir	Infra-red
Nmr	Nuclear magnetic resonance
ppm	Parts per million
TMS	Tetramethylsilane
DSS	Sodium 2,2-dimethyl-silapentane-5-sulphonate
GLC	Gas liquid chromatography
ip	Intraperitoneal
sc	Subcutaneous

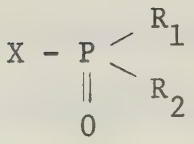
CHAPTER 1

INTRODUCTION

INTRODUCTION

The toxicity of organophosphorus compounds (referred to from now on as organophosphates) was first reported in the literature by the German chemist Willy Lange in 1932. Subsequently, a considerable amount of work in this field was carried out in Germany by Gerhard Schrader. His work led to the development of the fluorine-containing compounds including dyflos (di-isopropylfluorophosphonate, DFP), and the pyrophosphorus derivatives such as ethylpyrophosphate (tetraethyl-pyrophosphate, TEPP). This work had two direct results. Firstly, it led to the organophosphate insecticides in present day use, of which malathion (0,0'-dimethyl S-(1,2-dicarbethoxy ethyl) phosphorodithioate) and schradan (octamethylpyrophosphortetramide) are representative examples. Secondly, laboratories belonging to the armed forces began investigations into the possible application of compounds of this type as chemical warfare agents. Among the compounds considered sufficiently toxic for this purpose were ethylpyrophosphate, tabun (ethyl-N,N-dimethylphosphoramidocyanidate) and sarin (isopropylmethylphosphoro-fluoridate). The latter two compounds were produced on a large scale during the second world war.

Many thousands of organophosphates have been synthesized, Schrader alone prepared around 2000. However, a few general comments can be made here. All of these compounds are anticholinesterases - their mechanism of action will be discussed in more detail after consideration of the interaction between ACh and AChE. They are mainly pentavalent phosphorus compounds with the general structure:



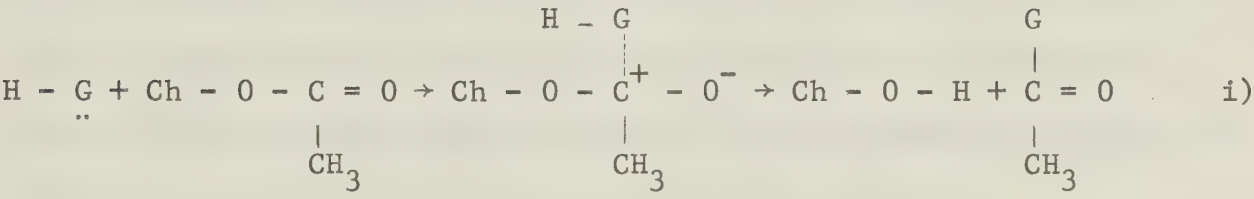
R_1 denotes an alkoxy group; R_2 is an alkoxy, alkyl or dialkylamido group; X is a halogen, cyanide, phenoxy or di-substituted phosphoryloxy group.

For convenience, it is possible to divide organophosphates of this general structure into two groups. On the one hand, compounds such as tabun or sarin are highly active anticholinesterase agents. In addition they are volatile and stable and so they are particularly suitable for employment as so-called nerve gases. On the other hand these are not very desirable properties for compounds which are to be used on a wide scale as insecticides in agriculture. Consequently, a second group of organophosphates are rather weak anticholinesterases, at least when compared with substances like sarin or tabun. However, their metabolites are highly active. An illustrative example is provided by malathion, which is typical of such low hazard pesticides. The selective toxicity to insects has been accounted for by differences in metabolism. Malathion is rapidly metabolized in mammals, principally by hydrolysis of the ethyl ester bonds, to give malathion monoester, whereas in insects oxidation to malaoxon is the principle route of metabolism. Malaoxon is some 1000 times more potent than malathion as an anticholinesterase. The endometatoxic action of Schradan provides another example of selective bioactivation.

A great deal of work has been done in order to clarify the interaction of ACh and AChE during the hydrolysis of ACh by the enzyme.

The groups in the ACh molecule which are most likely to be bound to the enzyme surface are the cationic head and the ester group (Wilson and Bergman 1950; Bergman et al 1950; Wilson et al 1950). That part of the enzyme surface which may bind the cationic head has been called the 'anionic site', and that which may bind the ester group the 'esteratic site' (Wilson and Bergman 1950).

Wilson et al (1950) have proposed a mechanism in which a basic group in the esteratic site of AChE forms a covalent bond with the carboxyl carbon atom of AChE:



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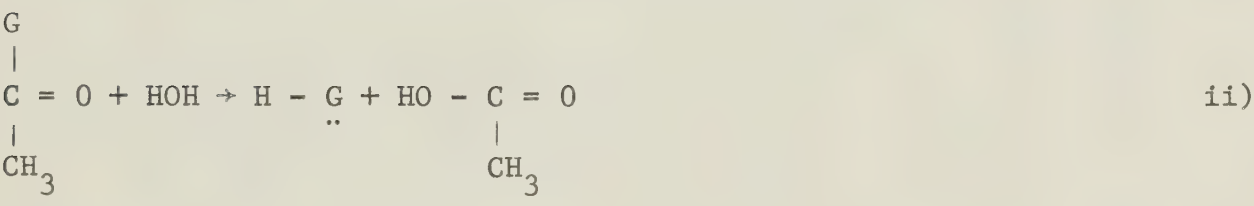


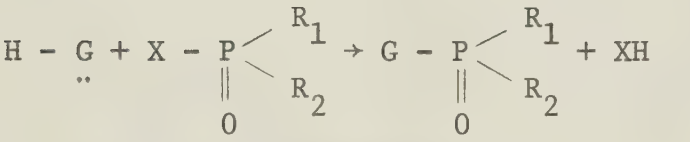
ii)

H – G is that part of the esteratic site of AChE which contains a basic group represented by the pair of unshared electrons and an acidic group, represented by the hydrogen atom. Ch is $CH_2CH_2N^+(CH_3)_3$.

Krupka and Laidler (1961) think it unlikely that a basic group forms the covalent bond and suggest that the oxygen atom of the hydroxyl group of a serine residue forms the bond, though they do not propose a complex involving a quadriligant carboxyl carbon atom. They have made a detailed proposal of the interaction of the enzyme and substrate, including the relative location of the reacting groups of

the enzyme and ACh. What appears likely (Triggle 1965) is that four groups in two sites of the enzyme are involved in the binding of AChE: the anionic site and an acidic group, a basic group and a serine residue of the esteratic site. The anionic site binds the trimethylammonium group of the substrate and may be a phosphoric acid group (Ariens 1962). The esteratic site binds and reacts with the acetyl group of the substrate. The basic group may be an imidazole nucleus (Krupka and Laidler 1961).

Poisoning by the organophosphate anticholinesterases has been shown to involve the "irreversible" inhibition of AChE. The result is that the normal enzyme function of hydrolyzing AChE is prevented. Wilson (1951) proposed that the reaction between organophosphates and AChE is analogous to the reaction between ACh and AChE:



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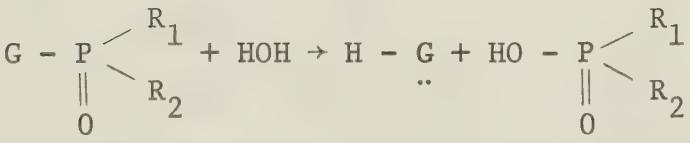


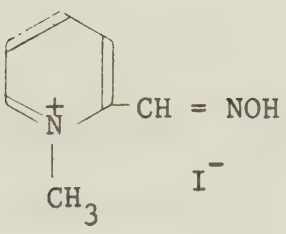
ii)

where H - G (G with two unshared electrons) is that part of the esteratic site of AChE which contains a basic group represented by the unshared electron pair, and an acidic group represented by the hydrogen atom. Phosphorylated AChE unlike acetylated AChE is extremely stable, i.e. reaction ii) proceeds at a very low rate, and for this reason organophosphates are inhibitors and

not substrates of AChE.

There is evidence that phosphorylation occurs at the serine hydroxyl group in AChE, (Schaffer et al 1953). This is not what would be expected from the mechanism of AChE proposed by Wilson et al (1950) or from the proposed reaction between AChE and organophosphates indicated above.

Wilson (1951) found that choline and hydroxylamine reactivated phosphorylated AChE at a much faster rate than water alone. Since, for purposes of therapy, a rapid return of activity is important, this early work led to the development of reactivators such as oximes or hydroxamic acids. Among these, pralidoxime iodide (2-pyridine aldoxime iodide, P-2-AM) (1) and NN'-trimethylene bis (4-hydroxyiminomethylpyridinium) dibromide (TMB-4) (2) are considered to be the most potent reactivators



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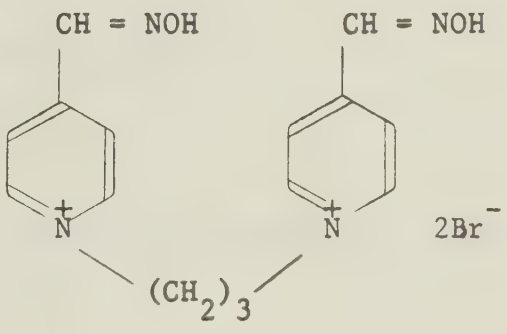
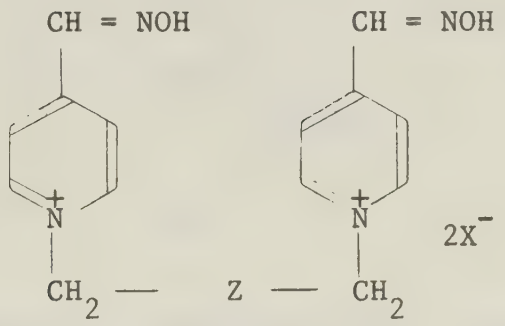


(2)

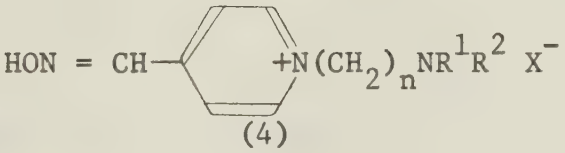
of phosphorylated AChE. A number of chemically related ethers, thioethers, sulfoxides and sulfones of general structure (3) in which Z is O, S, SO or SO_2 have been found to be suitable as antidotes for poisoning from organophosphorus compounds (Hauschild et al 1965). The chemically related diol of general structure (3) where Z = $(CHOH)_2$ was also found to be capable of preventing inhibition of AChE (Engelhard,

1964). Ashani and Cohen (1967) tested a series of heterocyclic oximes



(3)

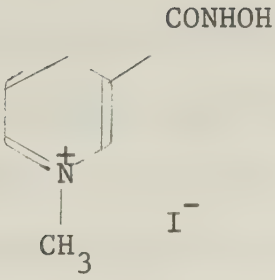
of general structure (4) as reactivators of phosphorylated cholinesterase. The most active member, 4-hydroxyiminomethyl-1-[3(N,N-dimethylamino)-n-propyl]-pyridinium chloride was found to possess excellent antidotal properties against intoxication with various organophosphorus insecticides. Nishimura et al (1967) have found



(4)

related diquaternary compounds to be efficient antidotes for organophosphate poisoning.

Hydroxamic acids are less active than oximes as reactivators of phosphorylated AChE but are more potent than hydroxylamine. The best hydroxamic acid reactivators are reported to be nicotinohydroxamic acid methiodide (5), pyrimidine-2-hydroxamic acid (6) and picolinohydroxamic acid (7) (Hobbiger 1963).



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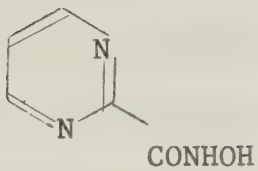

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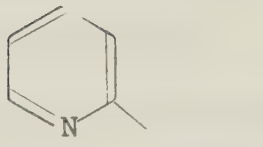
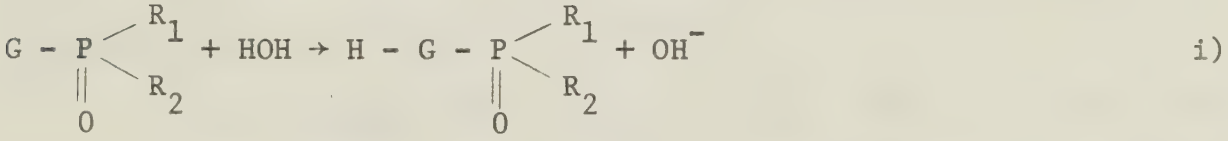


(7)

The active species of hydroxamic acids are their anions. The hydroxamate ions react with a protonated form of phosphorylated AChE. The ionizing group involved is assumed to be the acidic group in the esteratic site, which loses a proton during phosphorylation. Reactivation of phosphorylated AChE by hydroxamic acids, therefore, can be represented as follows (Hobbiger 1963):



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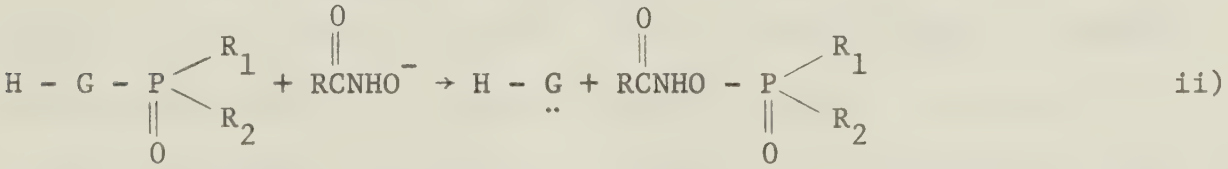
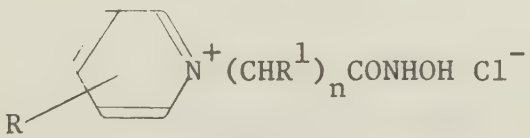


ii)

Coe (1959) found that quaternary hydroxamic acids derived from pyridine of general structure (8)



(8)

in which R is a hydrogen atom or an alkyl group and R^1 is a hydrogen atom (n = 1 or 2) or a methyl group (n = 1) were very effective in

preventing and reversing some of the physiological effects of cholinesterase inhibition. This observation prompted Coutts and his coworkers to extend their interest in hydroxamic acids to the preparation and pharmacological evaluation of compounds of general structural type (9)

$$RR^1N^+HCH_2CHR^2CONHOH\ Cl^-$$
(9)

in which the substituents R and R^1 were alkyl groups or a ring system and R^2 was a hydrogen atom or a methyl group. These compounds were envisaged as potential reactivators of phosphorylated AChE. However, preliminary pharmacological investigations revealed that some of these compounds possessed hypotensive activity.

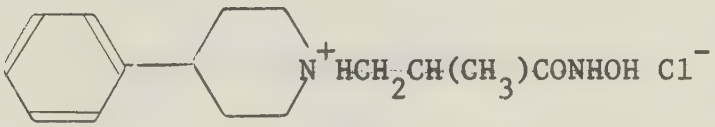
Coutts et al (1969) prepared methyl 3-aminopropionate hydrochlorides of general structure (10) and converted them to 3-aminopropionohydroxamic acid hydrochlorides of general structure (11) where R was a piperidino, piperazino, morpholino or homopiperidino group and R^1 was a methyl group or a hydrogen atom.

$$RCH_2CHR^1COOCH_3\ HCl$$
(10)

$$RCH_2CHR^1CONHOH\ HCl$$
(11)

The effects of intravenous administration of the ester hydrochlorides and the hydroxamic acid hydrochlorides on arterial blood pressure was

studied in anaesthetized cats. All of the hydroxamic acids and most of the esters caused a fall in the blood pressure to a varying degree. The duration of action of the hydroxamic acids was found to be more prolonged than that of the esters. Of the compounds studied, 2-methyl-3-(4-phenylpiperidino)propionohydroxamic acid hydrochloride (12), designated as K76, produced a marked and prolonged fall in the blood pressure.



(12)

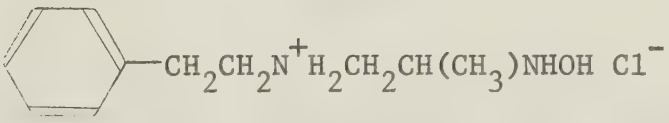
This observation led Midha *et al* (1970) to carry out a detailed pharmacological investigation to define the mechanism of K76-induced hypotension. These workers concluded that K76 produces a fall in blood pressure by blocking sympathetic ganglia. They found K76 to be as active as pentolinium tartrate on a molar basis.

Coutts *et al* (1969) showed that by changing the nature of the basic group R in compounds of general structure (10) and (11) the magnitude and duration of the fall in blood pressure produced by compounds of this type was significantly altered. Subsequently, Coutts *et al* (1971) extended these investigations to include compounds in which the group R was an alkyl or dialkylamino group as well as further compounds in which R was a piperidino or morpholino group. The group R^1 was an alkyl group or a hydrogen atom as before. The majority of the 3-aminopropionohydroxamic acid hydrochlorides and methyl 3-aminopropionate hydrochlorides examined produced a fall in the blood pressure

of anaesthetized cats. With certain compounds the hypotensive effect was prolonged, for example, 3-hexylaminopropionohydroxamic acid hydrochloride (13), 2-methyl-3-phenethylaminopropionohydroxamic acid hydrochloride (14) and methyl 2-methyl-3-dimethylaminopropionate hydrochloride (15). None of the compounds studied, however, was as active as 2-methyl-3-(4-phenylpiperidino)propionohydroxamic acid hydrochloride (K76) reported earlier (Midha et al 1970).

$$C_6H_{13}N^+H_2CH_2CH_2CONHOH\ Cl^-$$

(13)



(14)

$$(CH_3)_2N^+HCH_2CH(CH_3)COOCH_3\ Cl^-$$

(15)

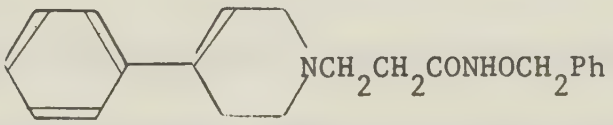
With the exception of the work of Coe and that of Coutts' group already outlined, very little work has been reported on the biological properties of aminohydroxamic acids of structural type (9). Gilbert et al (1961) have prepared a series of hydroxamic acids of general structure (16)

$$(CH_3)_3N^+(CH_2)_nCONHOH\ Cl^-\ n = 1 \text{ to } 3$$

(16)

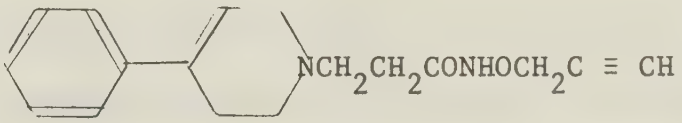
in an investigation of the relationship between structure and

reactivating ability among hydroxamic acid reactivators of phosphonate-inhibited AChE. None of these substances was found to be an effective reactivator, however, indicating that the reactivation reaction does not require a molecular configuration similar to ACh. One other group of compounds of related structure has been reported. Aralkoxy-amides of 4-phenyl-1,2,5,6-tetrahydropyridino alkanoic acids, such as (17) have been found to be useful in the treatment of hypertension and peripheral vascular disease (Biel, 1965).



(17)

These compounds produce a potent and prolonged blood pressure lowering which does not involve blockade of autonomic ganglia. The corresponding N-alkynyl- and N-alkynyloxy derivatives (18)

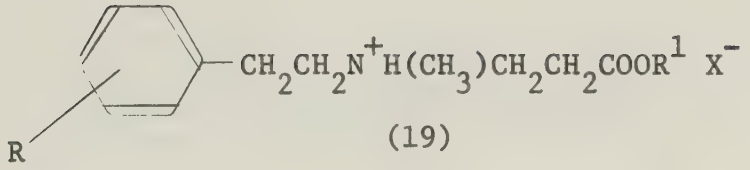


(18)

exhibit analgesic activity (Biel and Hopps, 1965).

As far as other pharmacological properties of amino-esters of general structural type (6) are concerned, rather more work had been reported in the literature than was the case with the amino-hydroxamic acids.

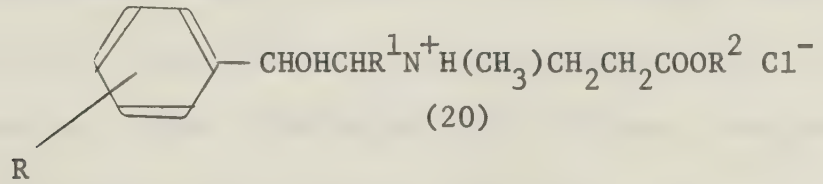
Baltzly et al (1949) prepared and tested a series of compounds of general structure (19) where R^1 was a methyl or ethyl group and X



(19)

was Cl or Br for oxytocic activity in analogy to lysergic acid. The maximum activity (5 - 10% of that shown by ergonovine per unit weight) was shown by compounds with two alkoxyl substituents on the phenyl ring. The monoalkoxy compounds had 0.1 of this activity and the compound with no substituents on the phenyl ring was inactive as were the phenolic compounds.

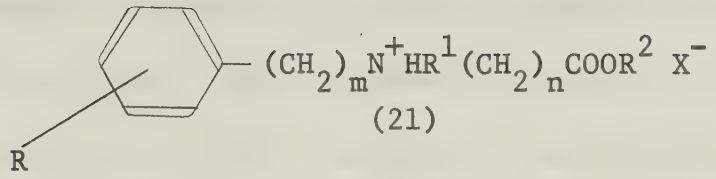
Baltzly and Phillips (1949[a]) studied the effect on oxytocic activity of introducing a hydroxyl group in the side chain in compounds of general structure (20).



(20)

The oxytocic activity of compounds with two alkoxy substituents on the phenyl ring, R^1 a hydrogen atom and R^2 a methyl or an ethyl group was not greater than that of the comparable substances without the hydroxyl group. On the other hand, monoalkoxy and phenolic compounds where R^1 was a methyl group and R^2 either a methyl or an ethyl group were also of the same order of activity, whereas in the phenethyl series maximum activity was observed only with two alkoxyl substituents on the aromatic ring.

Baltzly and Phillips (1949[b]) examined the effect on oxytocic activity of varying chain length in compounds of general structure (21).



(21)

The compound where R was 3,4-$(CH_3O)_2$, m was equal to 2, R^1 was a methyl group, n was equal to 1, R^2 was an ethyl group and X was Cl (21) together with similar compounds without any ring substituents did not prove to be of physiological interest. Since the homolog of (21) where n was equal to 2 was quite active, it was apparent to these investigators that at least two carbon atoms must intervene between the amino and carbalkoxyl groups. The reverse operation, i.e. increase of the distance between the amino and ester functions, produced no comparable change in physiological activity. Compounds with two alkoxy substituents on the phenyl ring, m equal to 2, R^1 a methyl group and n equal either to 3 or to 6 were found to be significantly less potent than their equivalents where n was equal to 2. It was concluded that there was no critical upper limit in this distance.

Phillips (1950) examined a number of compounds of general structure (22) i) bearing purely aliphatic substituents on the nitrogen;

$$RR^1N^+HCH_2CH_2COOCH_3\ Cl^-$$

(22)

ii) N-benzyl or N-phenethyl derivatives having not one but two 2-carboxy ethyl groups on the nitrogen; and iii) in which the nitrogen is part of a saturated heterocyclic ring. None of these compounds possessed significant oxytocic activity, but most of them were of low toxicity.

Numerous compounds of general structure (23) where R^1 and R^2 was a methyl group or a hydrogen atom and R was a variety of aliphatic or aromatic groups have been synthesized and tested for oxytocic activity by Horii *et al* (1962). Methyl 2-methyl-3-[2-(3-indolylethyl)] -propionate hydrochloride showed high oxytocic activity. Several other compounds of general structure (23) were also found to have oxytocic activity.

$$RR^1N^+CH_2CHR^2COOCH_3\ Cl^-$$
(23)

Of the possible compounds of general structural type (10) the reversed carboxyl analogue (24) bears the closest structural resemblance to ACh.

$$(CH_3)_3N^+CH_2CH_2COOCH_3\ I^-$$
(24)

It was synthesized by Bass *et al* (1950) who found it to be equipotent with ACh on the isolated guinea-pig ileum. Schueler and Keasling (1951) have extended these investigations to a number of compounds of related structure. Of particular interest are the reversed carboxyl analogues (25) and (26) of acetyl-α-methylcholine and acetyl-β-methyl-choline respectively.

$$(CH_3)_3N^+CH(CH_3)CH_2COOCH_3\ I^-$$
(25)

$$(CH_3)_3N^+CH_2CH(CH_3)COOCH_3\ I^-$$
(26)

These compounds were found to possess no more than 0.0001 of the activity of the normal analogues.

Matkovics et al (1961) and Pòrszàsz et al (1961) have examined a number of amino-esters of general structure (27) where

$$R(CH_2)_nCOOR^1 \quad n = 1 \text{ or } 2$$
(27)

R was a piperidino, pyrrolidino or morpholino group and R^1 a methyl, ethyl, butyl or benzyl group. Tertiary amino-esters and especially methyl esters were primarily nicotine-like in action. On the other hand, benzyl esters showed ganglionic blocking properties. The potency of these compounds was strongly influenced by the structure of the nitrogen containing ring as well as by the intramolecular distance between the nitrogen atom and the ester group. Unlike the tertiary analogues, the methiodides of piperidino and pyrrolidino acetyl esters displayed ganglionic blocking activity while those of piperidino, pyrrolidino and morpholino propionyl esters were nicotinic like the tertiary derivatives. The methiodide of methyl 3-pyrrolidylpropionate was found to augment glandular secretion and especially salivation without any marked cardiac effect. In this respect, this compound was more active than pilocarpine.

Barrass et al (1968) have also studied amino-esters of the same structural type (27) where R was a dimethylamino or pyrrolidino

group and R^1 was a methyl or an ethyl group. In addition, these

$$R(CH_2)_nCOOR^1 \quad n = 1 \text{ to } 4$$
(27)

investigators have examined a number of amino-amides of general structure (28) where R was a dimethylamino or pyrrolidino group as before and R^1

$$R(CH_2)_nCONR^1_2 \quad n = 1 \text{ to } 4$$
(28)

was a methyl group or a hydrogen atom. These amino-esters and amides were found to be predominantly nicotinic, but only the quaternary salts displayed appreciable activity. Although some of these compounds showed muscarinic activity they were not very potent in this respect, also the tertiary bases were much less active than the quaternary salts. During the course of this study Barrass and his co-workers re-evaluated the reversed carboxyl ester of ACh. They found it to have 0.16 the activity of ACh in contrast to the work of Bass et al (1950) who estimated it to be equipotent with ACh. Brimblecombe and Sutton (1968) have investigated the effects of some of these quaternary amino-esters (including methyl 3-dimethylaminopropionate methiodide and ethyl 3-dimethylaminopropionate methiodide) possessing both muscarinic and nicotinic activity on the cat superior cervical ganglion. These workers suggest that the amino-esters cause ganglion stimulation by interaction with postsynaptic, nicotinic and muscarinic receptors.

Various members of the series (29) and derivatives in which

certain of the CH_2 groups have alkyl substituents have been investigated by Halverstadt *et al* (1959). The quaternary ammonium groups were derived from lower aliphatic amines and heterocycles such as pyrrolidine, piperidine, morpholine and pyridine. A number of these compounds exhibited marked ganglionic blockade.

$$R_1R_2R_3N^+CH_2CH_2COOCH_2CH_2N^+R_4R_5R_6\ 2X^-$$
(29)

Apart from the work of Barrass *et al* (1968) very little has been reported concerning the biological properties of amino-amides of general structural type (28). Rosen *et al* (1956) have screened a number of propionamides for oxytocic activity. Of the compounds studied only one, namely, 3(2-ethanolamino)-N,N-diethylpropionamide (30) produced oxytocic activity. The inactive compounds included

$$HOCH_2CH_2NHCH_2CH_2CON(C_2H_5)_2$$
(30)

aliphatic substitutions, branched and straight chain, of 5 to 7 carbon atoms such as pentyl, isopentyl and 3,4-dimethoxyphenethyl and heterocyclic radicals such as morpholino and piperidino combined with aralkyl radicals. Surrey and Olivet (1963) have found a number of quaternary ammonium salts of the N-tertiary aminoalkyl tertiary amino alkanamides, of which N-(2-dibutylaminoethyl)-3-dibutylaminopropionamide dimethiodide (31) is a representative example, to have ganglionic blocking activity.

$$(C_4H_9)_2N^+(CH_3)CH_2CH_2CONHCH_2CH_2(CH_3)N^+(C_4H_9)_2\ 2I^-$$
(31)

A number of amino-esters and amides structurally related to those discussed so far were screened for antispasmodic activity by Pacheo et al (1962). The esters were of general structure (32) where R was a piperidino, pyrrolidino or morpholino group, R^1 was usually a phenyl

$$RCHR^1CH_2COOR^2\ HCl$$
(32)

group and R^2 a methyl, ethyl or higher group. The amino-amides were of general structural type (33) where R was a piperidino, pyrrolidino

$$RCHR^1CH_2CONHR^2$$
(33)

or morpholino group, R^1 a phenyl group and R^2 usually a phenyl or substituted phenyl group. Of the compounds examined, butyl 3-phenyl-3-pipiridylpropionate hydrochloride and isobutyl 3-phenyl-3-pyrrolidyl-propionate hydrochloride showed high antispasmodic activity and low toxicity.

Many of the amino-esters of general structure (34) were prepared by the interaction of methyl acrylate or methyl methacrylate and an appropriate amine (Baltzly et al 1949; Baltzly and Phillips 1949[a]; Phillips 1950; Pacheo et al 1962; Horii 1962; Barrass et al 1968; Coutts et al 1969; 1971):

$$RR^1NH + CHR^2 = CR^3COOR^4 \rightarrow RR^1NCHR^2CHR^3COOR^4 \quad (34)$$

Since this method has been employed in the present study to synthesize compounds of this type, it is convenient to discuss at this point the pertinent literature.

Phillipi and Galter (1929) studied the reaction of ammonia and amines with several unsaturated esters. These investigators found that the main product of the reaction of methylamine with ethyl crotonate was 3-methylaminobutyric methylamide, only a small amount of ethyl 3-methylaminobutyrate being obtained. By contrast, the only product of the reaction with piperidine was ethyl 3-piperidinobutyrate. No reaction was found to occur between ethyl 2-ethylacrylate and either liquid ammonia or methylamine. The interaction of ethyl 3-dimethylacrylate and ammonia gave about 60% of the addition product but no amide; reaction with methylamine gave some ethyl 3-methylaminoisovalerate but the main product was the methylamide. Piperidine did not react with ethyl 2-ethylacrylate.

Morsch (1932; 1933) has studied the action of ammonia, methylamine and diethylamine on ethyl crotonate, ethyl cinnamate and methyl acrylate respectively. This investigator found the products formed in these reactions to be dependent upon i) the relative amounts of the two reactants; ii) the duration of the reaction; iii) the reaction temperature and iv) the use of a solvent in the reaction. For example, methyl acrylate (1 mol) and 10% methanol-methylamine (0.55 mol) when allowed to react for two days at room temperature gave 22% methyl

3-methylaminopropionate (35) and 70% of dimethyl 3,3'methylimino-dipropionate (36); 1 mol methyl acrylate and 1.1 mols of methylamine for 2 days gave 24% (35), 35% (36) and 5.5% of 3-methylaminopropionic methylamide (37); 1 mol methyl acrylate and 4 mols of methylamine for 4 days gave 64% (37). With 1 mol methyl acrylate and 1.1 mols of methylamine in absolute ethanol left at room temperature for 2 days there resulted 39.5% (35), 32% (36) and 4% (37). Using 1 mol methyl acrylate and liquid methylamine the following results were obtained on heating 8 hours at 60 - 65°: 0.55 mol methylamine, 5% (35) and 89.5% (36); 1.1 mols methylamine, 31% (35), 36.5% (36) and 9% (37); 4 mols methylamine, 71.5% (37). Methyl acrylate and diethylamine interacted when allowed to stand for several days at room temperature or refluxed for 1 hour and gave methyl 3-diethylaminopropionate; heating methyl acrylate with 3 mols of diethylamine for 24 hours at 190 - 200° gave 3-diethylamino-propionic diethylamide.

Bieber (1954) has shown the ease of addition of certain amines to methyl methacrylate to be in the order piperidine > diethylamine > aniline > phenylethylamine. Methyl methacrylate treated with piperidine gave methyl 2-methyl-3-piperidinopropionate. In a sealed tube, for 15 hours at 120°, diethylamine and methyl methacrylate interacted and yielded methyl 2-methyl-3-diethylaminopropionate. When methyl methacrylate stabilized with 0.1% hydroquinone was heated 60 hours with aniline the amino-ester was obtained in relatively low yield while methyl methacrylate heated 87 hours with phenylethylamine gave no amino-ester.

In general amines react with the lower aliphatic esters and nitriles of acrylic and methacrylic acid and crotonic acid without the aid of a catalyst. In this addition reaction steric effects are very important, however, and the size and branching of the alkyl groups in the amine have a definite influence on the reaction. Luskin et al (1956) found the yields obtained in the addition of n-butylamine, tert-butylamine and 1,1,3,3-tetramethylbutylamine to methyl methacrylate were 58% methyl 2-methyl-3-n-butylaminopropionate (38), 26% methyl 2-methyl-3-tert-butylaminopropionate (39) and 14% methyl 2-methyl-3-(1,1,3,3-tetramethylbutylamino)propionate (40) respectively.

$$CH_3(CH_2)_3NHCH_2CH(CH_3)COOCH_3$$
(38)

$$(CH_3)_3CNHCH_2CH(CH_3)COOCH_3$$
(39)

$$(CH_3)_3CCH_2C(CH_3)_2NHCH_2CH(CH_3)COOCH_3$$
(40)

Taylor et al (1959) found that n-butylamine was readily cyanoethylated in 85 - 95% yield to give 3-n-butylaminopropionitrile (41), tert-butylamine was cyanoethylated only in 4.8 - 5.6% yield to give 3-tert-butylaminopropionitrile (42) and 1,1,3,3-tetramethylbutylamine gave no yield of 1,1,3,3-tetramethylbutylaminopropionitrile (43).

$$CH_3(CH_2)_3NHCH_2CH_2CN$$
(41)

$$(CH_3)_3CNHCH_2CH_2CN$$
(42)

$$(CH_3)_3CCH_2C(CH_3)_2NHCH_2CH_2CN$$
(43)

Luskin et al (1956) have described yield improvements in reactions of tert-carbinamines with methyl methacrylate and acrylonitrile by using acidic conditions. The addition of small amounts of hydrochloric acid to the reaction mixture increased the yields obtained from the addition of tert-butylamine and 1,1,3,3-tetramethylbutylamine to methyl methacrylate to 42% of (42) and 33% of (43) respectively. Yield improvements in cyanoethylation were obtained by using dilute aqueous acetic acid as a catalyst. In this fashion the yield of the cyanoethylated derivative of tert-butylamine (42) was increased from about 5 to 89% and the yield using 1,1,3,3-tetramethylbutylamine was raised from 0 to 83% of (43).

Taylor et al (1959) have found that these other supposed catalysts are not needed for these yield increases because water alone catalyzes the cyanoethylation of tert-carbinamines. The yields were always substantially zero when anhydrous reactants were used and ranged from 95.5% for tert-butylamine, to 27.8% for tert-nonylamine when water was added. The solubility of water in the amines used in this study decreased rapidly as the molecular weight of the amine increased. While this lack of solubility in the higher amines resulted in lower conversions, the addition of N,N-diethylformamide which brought about

homogeneity of the reacting system, but was non-catalytic itself in the cyanoethylation reaction, restored the catalytic effect of the water.

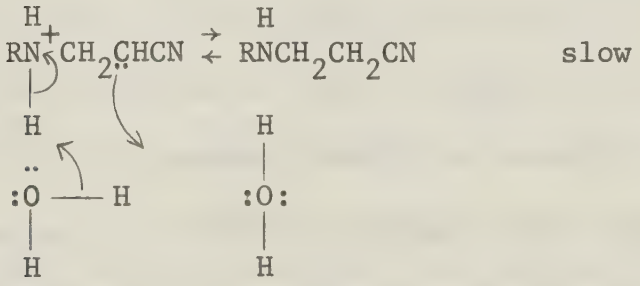
The mechanism of the cyanoethylation reaction has been postulated as attack by an anion or available electron pair at the terminal carbon atom of acrylonitrile followed by loss and gain of protons as needed to complete the reaction (Burston 1949; Ogato et al 1956; Zellars and Levine 1948). Thus in the case of amines the mechanism has been postulated as:

$$RCH_2NH_2 + CH_2 = CHCN \rightleftarrows RCH_2N^+H_2CH_2CHCN \quad \text{slow}$$

$$RCH_2N^+H_2CH_2CHCN \rightleftarrows RCH_2NHCH_2CH_2CN \quad \text{fast}$$

According to Taylor and his co-workers the concluding step, previously considered the fast step, may, because of steric factors, be the rate-determining step and the effect of water in catalysis is in the role of a proton transfer agent effecting completion of the reaction:

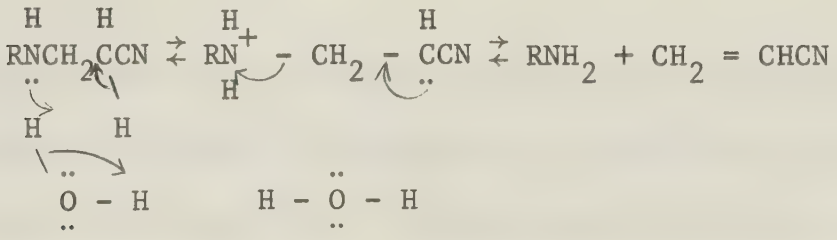
$$\begin{matrix} H \\ RN: \\ H \end{matrix} + CH_2 = CNCN \rightleftarrows \begin{matrix} H \\ RN^+ \\ H \end{matrix} CH_2\ddot{C}HCN \quad \text{fast}$$



For a compound to act as a proton transfer agent under these conditions it must have an unshared pair of electrons with a labile hydrogen atom

on the same atom which contains the unshared pair and the geometry of the proton transfer agent must allow it to physically fit the cyanoethylation intermediate. Thus unhindered amines, alcohols, amides, thiols and the like should be effective in such a role. In the cyanoethylation of unhindered amines, excess of unreacted amine itself can satisfactorily act as a transfer agent leading to high yields of product. In the case of highly hindered amines, such as the tert-carbinamines, the steric hindrance around the amino group prevents it from approaching the similarly hindered cyanoethylation intermediate to allow completion of the reaction. These effects become obvious only in essentially anhydrous reaction systems. The amounts of water usually found in the reagents used in these reactions are enough to promote cyanoethylation.

If the proposed role of water in this system is correct, a similar effect should be found in the reversal of cyanoethylation:



Considering the fact that proton transfer would again be necessary to effect reversal of the reaction it would be expected that pure anhydrous amine cyanoethylation products would not readily revert to acrylonitrile and amine. In order to evaluate this hypothesis experimentally, Taylor and his co-workers studied the reversal of amine cyanoethylation. It was found that reversal of reaction did not

occur when the anhydrous 3-alkylaminopropionitriles were heated, but when heated in the presence of water, appreciable reversal of cyanoethylation to give free amines did occur.

Taylor's group concluded that certain other compounds containing the required structure, i.e. a labile hydrogen atom attached to an atom which also has a free pair of electrons, should act as catalysts for cyanoethylation of sterically hindered amines. Of particular interest was the fact that the effectiveness of alcohols as catalysts decreased as the molecular complexity of the alcohol increased. That this decrease was not merely a concentration effect was shown by runs in which equimolar amounts of n-propyl-, isopropyl- and tert-butyl alcohols were used as catalysts. According to the proposed mechanism, this effect was the result of increasing difficulty in the alcohol approaching the intermediate to act as a proton transfer agent to complete the reaction.

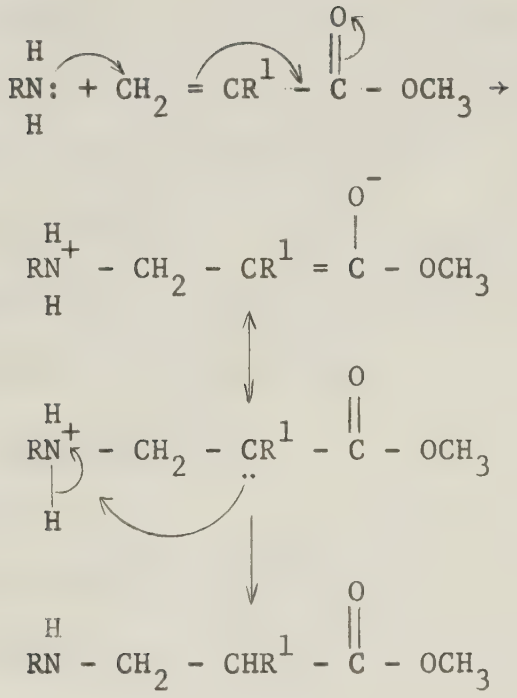
A comparison of the dielectric constants of compounds employed as catalysts failed to disclose any trend which might be affected by dielectric character. No other characteristics of the materials used as catalysts were found to be directly relatable to their catalytic activity. Thus, the proposed mechanism was considered to fit the facts more closely than any proposal based purely on solvent effects.

Hughes (1960) found that methyl methacrylate did not react when refluxed with dry tert-butylamine, but gave 13.9% conversion to methyl 2-methyl-3-tert-butylaminopropionate (39) with 12% water added

to the reaction mixture. Also methyl crotonate was reacted with 3-cyclohexyl-3-aminopentane in the presence of 0.45 mol of water to give a good yield of ethyl-3-methyl-4-aza-5-ethyl-5-cyclohexyl-heptanoate. The same reaction between the dry amine and the dry ester in which no water was added to the reaction mixture did not yield any appreciable quantity of the heptanoate.

Montgomery and Hughes (1961) have treated tertiary carbinamines of the type $RR^1R^2CNH_2$ where R, R^1 and R^2 were alkyl, cycloalkyl, aryl, alkaryl and aralkyl radicals containing 1 to 12 carbon atoms, with esters and nitriles of lower α,β-unsaturated acids in the presence of water and an N,N-disubstituted amide solubilizer to yield the corresponding 3-aminonitriles and 3-aminoesters. Maximum yields were obtained with 3 - 10% water by weight of amine and a minimum amount of solubilizer which effected a homogeneous solution of the reactants and water.

The mechanism of the Michael addition of primary amines to acrylates can be postulated (Pfau 1967) as illustrated:



Sanui and Ogata (1967) have examined the catalytic accelerating effect of active hydrogen compounds such as n-butyl alcohol, sec-butyl alcohol, tert-butyl alcohol, ethanol, water and n-butyl mercaptan on the rate of addition of n-butylamine to ethyl acrylate; the accelerating effect of the mercaptan was particularly large, the rate being 350 times as fast as in the absence of mercaptan. It was found by GLC of the reaction products that the amount of n-butylamine kept constant all through the reaction, showing that amine accelerates the addition of the mercaptan to ethyl acrylate. Even a small amount of n-butylamine accelerated the addition reaction very rapidly, showing that the mercapto group was more nucleophilic than amine.

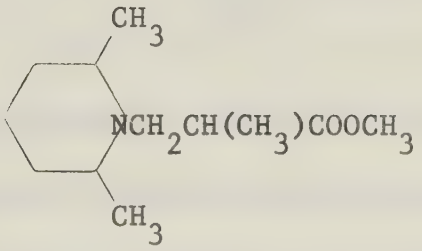
It has been observed by Howton (1945) that esters of acrylic acid exhibit a greater tendency to combine with primary and secondary amines than do the corresponding esters of methacrylic acid. Aniline

reacted with methyl acrylate to give methyl 3-phenylaminopropionate, while under the same conditions no reaction was obtained with methyl methacrylate; similarly di-n-butylamine could be added to ethyl acrylate but not to ethyl methacrylate. Pollard et al (1953) found methyl and ethyl methacrylates did not react with either 1-phenylpiperazine or 1-(2-methylphenyl)-piperazine under conditions which were successful with unsubstituted acrylate esters.

The synthesis by Coutts et al (1969; 1971) of various methyl 3-aminopropionates has already been mentioned. With two exceptions, these investigators found monoalkylamines and dialkylamines with unbranched alkyl chains reacted readily with methyl acrylate and methyl methacrylate in the absence of basic or acidic catalysts to give good yields of 3-aminoesters. The exceptions were di-n-propylamine and phenethylamine which reacted only slowly with methyl methacrylate. Attempts to react di-isopropylamine with methyl methacrylate in boiling methanol or n-butanol for lengthy periods of time were unsuccessful and only starting materials were recovered. These results were in agreement with the findings of Luskin et al (1956) but were in contrast with the claims of Suminov (1967) who reported the synthesis of methyl 2-methyl-3-di-isopropylaminopropionate in 32.5% yield by boiling a mixture of di-isopropylamine and methyl methacrylate in methanol for 24 hours.

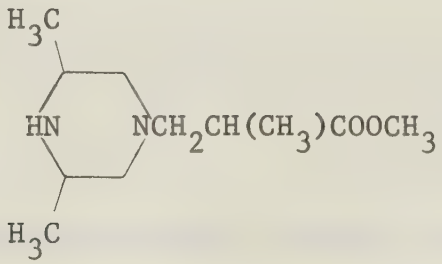
Suminov (1967) has also claimed a successful synthesis of methyl 2-methyl-3-(2,6-dimethylpiperidino)propionate (44) in 70% yield from methyl methacrylate and 2,6-dimethylpiperidine. Coutts' group were

unable to repeat this reaction using either the same or more vigorous conditions.



(44)

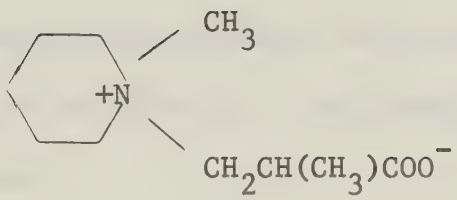
These investigators also found that when methyl methacrylate (2 mols) and 2,6-dimethylpiperazine (1 mol) were heated under reflux in methanol, the only product was methyl 2-methyl-3-(2,6-dimethyl-4-piperazinyl)-propionate (45),



(45)

due to steric hindrance preventing reaction at the nitrogen atom with two methyl groups on adjacent carbon atoms.

According to Vystrcil and Hudeck (1950) when aliphatic amines were reacted with methyl methacrylate in an aqueous medium, a shift of the ester methyl group resulted in the formation of N-methyl substituted betaines such as (46)



(46)

Coutts et al (1970) reacted methyl methacrylate with piperidine in the presence of water using the conditions described by Vystrcil and Hudeck (1950). The product was methyl 2-methyl-3-pipiridylpropionate, no betaine being obtained.

The amino-amides of general structure (33) screened by Pacheo's group (1962) for antispasmodic activity were prepared by refluxing excess piperidine, pyrrolidine or morpholine with the appropriate α,β-unsaturated amide:

$$RNH + R^1CH = CH_2CONHR^2 \rightarrow RCHR^1CH_2CONHR^2$$

(33)

Compared to the substantial volume of information relating to the Michael-type addition of amines across the double bond of the lower aliphatic esters of α,β-unsaturated acids, however, very little work has been reported concerning the addition of amines to α,β-unsaturated amides. Erickson (1952) has prepared several 3-dialkylaminopropionamides (47) by the addition of secondary aliphatic amines to acrylamide:

$$R_2NH + CH_2 = CHCONH_2 \rightarrow R_2NCH_2CH_2CONH_2$$

(47)

Dimethylamine, dipropylamine, dibutylamine and morpholine were found to react readily in alcohol solution at room temperature giving good yields of the amino-amides.

Suminov (1967) has studied the influence of various solvents and catalytic mixtures on the rate of addition of piperidine, hexamethyleneimine, benzylamine, n-butylamine, hydrazine hydrate, 3,5,5-trimethylpyrazoline and 3,5-dimethylpyrazole to acrylamide to yield the corresponding 3-alkyl- and 3-dialkylaminopropionamides. Hexamethyleneimine, n-butylamine, and hydrazine hydrate were the most active while 3,5,5-trimethylpyrazoline and 3,5-dimethylpyrazole reacted only after prolonged boiling in water. The addition of amines to acrylamide took place most easily in water, worst in alcohols whilst in aprotic solvents the reaction rate decreased. Proton donors such as ammonium chloride and boric acid increased the reaction rate of the addition of 3,5,5-trimethylpyrazoline to acrylamide, while the presence of sodium fluoride, potassium fluoride, sodium acetate and potassium acetate did not affect the addition.

CHAPTER 2

AIMS AND OBJECTS

AIMS AND OBJECTS

It is evident from the preceding introduction that changes in the nature of the basic group R in compounds of general structure (10) and (11) can significantly affect their pharmacological activity.

$RCH_2CHR^1COOCH_3$ HCl (10) $RCH_2CHR^1CONHOH$ HCl (11)

Among the large number of structures of this type evaluated as hypotensive agents by Coutts and co-workers (1971) were a series of 3-monoalkylaminopropionohydroxamic acids together with their methyl ester precursors:

i) 3-Monoalkylaminopropionohydroxamic acids (Coutts et al 1971)

$RN^+H_2CHR^1CONHOH\ Cl^-$ (48)

Compound	R	R^1
49	CH_3	H
50	CH_3CH_2	H
51	$CH_3(CH_2)_2$	H
52	$CH_3(CH_2)_2$	CH_3
53	$CH_3(CH_2)_3$	H
54	$CH_3(CH_2)_5$	H

ii) Methyl 3-monoalkylaminopropionates (Coutts et al 1971)

$$RN^{+}H_2CH_2CHR^{1}COOCH_3\ Cl^{-}$$

(55)

Compound	R	R^1
56	CH_3	H
57	CH_3CH_2	H
58	$CH_3(CH_2)_2$	H
59	$CH_3(CH_2)_2$	CH_3
60	$CH_3(CH_2)_3$	H
61	$CH_3(CH_2)_5$	H

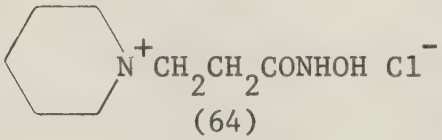
These investigators found that with the exception of 3-methylpropionohydroxamic acid hydrochloride (49) which caused a rise, all of these 3-monoalkylaminopropionohydroxamic acid hydrochlorides produced a fall in the blood pressure of anaesthetized cats. The most active compound in this respect was 3-hexylpropionohydroxamic acid hydrochloride (54) in terms of both the magnitude and the duration of its hypotensive effect. All the corresponding methyl 3-monoalkylaminopropionates possessed a hypotensive action save one, namely, methyl 3-propylaminopropionate hydrochloride (58) which produced a rise in the blood pressure. Once again, activity was found to be maximal when the alkyl chain of the amine part of the compound was hexyl, i.e. methyl 3-hexylaminopropionate hydrochloride (61).

The presence of a methyl substituent in the 2-position in 2-methyl-3-propylaminopropionohydroxamic acid hydrochloride (52) was found to result in a decreased hypotensive activity of this compound as compared to its unbranched analogue, 3-propylaminopropionohydroxamic acid hydrochloride (51). An opposite effect of methyl substitution in the 2-position was observed however, in the dialkylamino compounds 3-dimethylaminopropionohydroxamic acid hydrochloride (62) and 2-methyl-3-dimethylaminopropionohydroxamic acid hydrochloride (63) prepared in the same study.

$$(CH_3)_2N^+HCH_2CH_2CONHOH\ Cl^-$$
(62)

$$(CH_3)_2N^+HCH_2CH(CH_3)CONHOH\ Cl^-$$
(63)

The branched compound (63) was more active than the unbranched analogue (62) particularly as regards the duration of its hypotensive effect. The same phenomenon was also observed with two other compounds synthesized during the course of this investigation, namely 3-piperidinopropionohydroxamic acid hydrochloride (64) and 2-methyl-3-piperidinopropionohydroxamic acid hydrochloride (65)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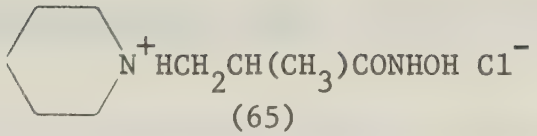


(65)

The magnitude and duration of the fall in the blood pressure produced by the branched hydroxamic acid (65) was greater than that of the unbranched compound (64).

It was thought desirable, therefore, to investigate an extended series of 3-monoalkylaminopropionohydroxamic acids (48) both to observe general structure-activity relationships and to identify the optimum chain length necessary for significant hypotensive activity.

Accordingly, investigation of the following structures was proposed:

3-Monoalkylaminopropionohydroxamic acids

(48)

Compound	R	R^1
66	CH_3	CH_3
67	CH_3CH_2	CH_3
68	$CH_3(CH_2)_2$	CH_3
69	$CH_3(CH_2)_4$	H
70	$CH_3(CH_2)_4$	CH_3
71	$CH_3(CH_2)_5$	CH_3
72	$CH_3(CH_2)_6$	H

Continued

3-Monoalkylaminopropionohydroxamic acids (Continued)

Compound	R	R^1
73	$CH_3(CH_2)_6$	CH_3
74	$CH_3(CH_2)_7$	H
75	$CH_3(CH_2)_7$	CH_3
76		H
77		CH_3
78		H
79		CH_3

In the event, not all of these structures were prepared during the course of this work and numerous others were; the reasons for making them are discussed at appropriate points in the text.

Finally, it was noted that Coe (1959) and Gilbert et al (1961) have prepared quaternary hydroxamic acids closely related to the structures proposed in this study and evaluated them as protectors of AChE from the inhibiting effects of organophosphates. Therefore, another aim of the work was to prepare potential antidotes for organophosphate poisoning.

CHAPTER 3

SYNTHESIS OF 3-MONOALKYLAMINOPROPIONO-HYDROXAMIC ACIDS AND METHYL 3-MONOALKYLAMINOPROPIONATES

SYNTHESIS OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS AND METHYL 3-MONOALKYLAMINOPROPIONATES

i) SYNTHESIS OF METHYL 3-MONOALKYLAMINOPROPIONATES

In order to obtain 3-monoalkylaminopropionohydroxamic acids of general structure (48), it was necessary to synthesize various methyl 3-monoalkylaminopropionates (80). This was accomplished by the Michael addition of the appropriate primary amine to the double bond of methyl acrylate or methyl methacrylate:

$$CH_2 = CR^1COOCH_3 + RNH_2 \rightarrow \underset{(80)}{RNHCH_2CHR^1COOCH_3}$$

The following amino-ester precursors (80) were prepared during the course of the present work:

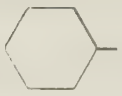
Methyl 3-monoalkylaminopropionates

$$\underset{(80)}{RNHCH_2CHR^1COOCH_3}$$

Compound	R	R^1
81	CH_3	CH_3
82	CH_3CH_2	CH_3
83	$CH_3(CH_2)_2$	CH_3
84	$(CH_3)_2CH$	H
85	$(CH_3)_2CH$	CH_3

Continued

Methyl 3-monoalkylaminopropionates (Continued)

Compound	R	R^1
86	$CH_3(CH_2)_3$	CH_3
87	$CH_3CH_2CHCH_3$	H
88	$CH_3CH_2CHCH_3$	CH_3
89	$(CH_3)_3C$	H
90	$(CH_3)_3C$	CH_3
91	$CH_3(CH_2)_4$	H
92	$CH_3(CH_2)_4$	CH_3
93		H
94		CH_3
95	$(CH_3)_2CH(CH_2)_2$	H
96	$(CH_3)_2CH(CH_2)_2$	CH_3
97	$CH_3(CH_2)_5$	CH_3
98		H
99		CH_3
100	$CH_3(CH_2)_6$	H
101	$CH_3(CH_2)_6$	CH_3
102	$CH_3(CH_2)_7$	H
103	$CH_3(CH_2)_7$	CH_3
104	$(CH_3)_3CCH_2C(CH_3)_2$	H
105	$(CH_3)_3CCH_2C(CH_3)_2$	CH_3
106	$CH_2 = CHCH_2$	H
107	$CH_2 = CHCH_2$	CH_3

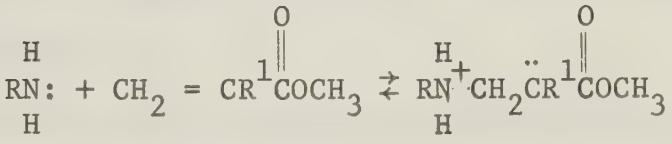
Two sets of conditions were employed in the Michael addition of the appropriate amine to the double bond of methyl acrylate or methyl methacrylate. With the higher straight chain amines the condensation proceeded smoothly when the reactants were refluxed together in anhydrous methanol for a number of hours. In the case of the lower straight chain amines, branched amines and cyclic amines, the reactants were dissolved in anhydrous methanol and allowed to stand at room temperature for varying periods of time (Ziering et al 1947).

The choice of methanol as a reaction solvent arose from several considerations. The information available, from several literature sources, suggested that methanol might act catalytically in these reactions. Sanui and Ogata (1967) demonstrated a catalytic accelerating effect of certain active hydrogen compounds such as, n-butyl alcohol, sec-butyl alcohol, tert-butyl alcohol, ethanol, water and n-butyl mercaptan on the rate of addition of n-butylamine to ethyl acrylate. Sanui et al (1968) observed that in the addition of diamines to ethyl acrylate the rate in methanol solution was about 100 times as fast as in tetrahydrofuran solution.

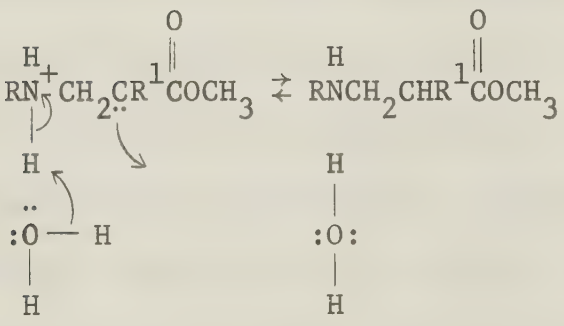
Taylor et al (1959) showed that the reversible cyanoethylation of tert-carbinamines with acrylonitrile was catalyzed by water or compounds having a labile hydrogen atom attached to an atom that also had a free pair of electrons. An excess of sterically unhindered amine was itself a catalyst for cyanoethylation. The catalytic accelerating effect of water on the reaction of sterically hindered tert-carbinamines with esters of α,β-unsaturated acids has been demonstrated by

Hughes (1960) and Montgomery and Hughes (1961). In view of the findings of Bieber (1954) and Coutts *et al* (1971), however, concerning the ease with which 3-monoalkylaminopropionates form the corresponding amino acids in the presence of water, it was thought undesirable to employ water as a catalyst in these preparations.

The mechanism of catalysis in these reactions may be the same as that proposed by Taylor *et al* (1959) for the cyanoethylation of *tert*-carbinamines, namely, an attack by an electron pair at the terminal carbon atom followed by a rate-determining proton transfer to complete the reaction:



fast



slow

Such a mechanism can explain why hindered amines undergo addition to the lower esters of α,β-unsaturated acids with much more difficulty than do unhindered amines (Luskin *et al* 1956; Hughes 1960). Unhindered amines should themselves catalyze reactions of this type by acting as proton transfer agents. In the case of highly hindered amines, however, steric hindrance around the amino group makes approach to the similarly

hindered intermediate difficult.

Sanui and Ogata (1967) found the most effective of the three butyl alcohols in catalyzing the addition of n-butylamine to ethyl acrylate was the straight chain n-butyl alcohol. The most highly branched of the butyl alcohols, tert-butyl alcohol, was the least effective of the three, sec-butyl alcohol lying somewhere in between. This effect can be explained as the result of increasing difficulty in the alcohol approaching the intermediate to act as a proton transfer agent to complete the reaction. Taylor et al (1959) observed a similar decrease in the effectiveness of alcohols in the catalysis of the cyanoethylation of tert-butylamine as the molecular complexity of the alcohol increased.

An important consideration taken into account in selecting methanol as a solvent for the reactions described in this thesis was the desirability of avoiding any possibility of ester exchange taking place. When ethyl acrylate and piperidine were boiled under reflux in methanol, instead of the expected ethyl 3-piperidinopropionate, the corresponding methyl ester (108) was obtained. A transesterification reaction apparently occurred under the conditions employed in this reaction. Baltzly et al (1949) have used this type of reaction in transforming methyl amino-ester hydrochlorides of general structure (19) into higher esters. However, it would appear that this phenomenon is not always observed in reactions of this type. For example, Ganellin and Spickett (1965) have prepared methyl 2-methyl-3-benzylaminopropionate in 66% yield by heating a solution of benzylamine and

methyl methacrylate in ethanol for 6 hours. Casy *et al* (1968) synthesized methyl 2-methyl-3-allylaminopropionate by stirring a mixture of allylamine and methyl methacrylate in ethanol for 6 hours and then refluxing for 16 hours. Nevertheless, since ester exchange was observed when ethyl acrylate and piperidine were refluxed in ethanol, it was thought advisable to employ methanol as the solvent of choice in reactions involving methyl acrylate or methyl methacrylate.

Using the procedures already outlined, the various methyl 3-monoalkylaminopropionates were obtained in yields which ranged between 22 and 70% of the theoretical.

Of particular interest was the addition of sterically hindered *tert*-carbinamines to methyl acrylate and methyl methacrylate. When *tert*-butylamine and methyl acrylate were reacted for 6 weeks at room temperature, methyl 3-*tert*-butylaminopropionate (89) was obtained in 56% yield. In the addition of *n*-butylamine to methyl methacrylate, methyl 2-methyl-3-*n*-butylaminopropionate (86) was obtained in 40% yield after 16 hours at room temperature. When *tert*-butylamine and methyl methacrylate were reacted for 6 weeks at room temperature, methyl 2-methyl-3-*tert*-butylaminopropionate (88) was obtained in 42% yield. The steric hindrance offered by the bulky *tert*-butyl group in this case can be clearly seen by comparing the reaction times employed and the corresponding yields obtained. Luskin *et al* (1956) obtained yields of 58% and 26% respectively on refluxing *n*-butylamine and methyl methacrylate for 6 hours and *tert*-butylamine and methyl methacrylate for 48 hours. Hughes (1960) obtained a conversion of 13.9% by

refluxing tert-butylamine and methyl methacrylate together with 12% water for 6 hours. No reaction was observed when the synthesis was repeated using anhydrous reagents without a catalyst. In the addition of 1,1,3,3-tetramethylbutylamine to methyl methacrylate, methyl 2-methyl-3-(1,1,3,3-tetramethylbutylamino)propionate (105) was obtained in 32% yield after 7 weeks at room temperature. Luskin et al (1956) obtained a yield of 14% in the addition of 1,1,3,3-tetramethylbutylamine to methyl methacrylate by heating the reactants for 48 hours. The reaction of 1,1,3,3-tetramethylbutylamine with methyl acrylate gave methyl 3-(1,1,3,3-tetramethylbutylamino)propionate (104) in 57% yield after 7 weeks at room temperature.

Under the reaction conditions employed in this study methanol appeared to be an effective catalyst in the addition of sterically hindered tert-carbinamines to methyl acrylate and methyl methacrylate. The yields obtained in the addition of sec-butylamine to both methyl acrylate and methyl methacrylate after 7 weeks at room temperature were only 38 and 39% respectively in spite of the long reaction period. In contrast, the yield in the addition of isopropylamine to methyl acrylate was 67% although in the addition of isopropylamine to methyl methacrylate it was 44%.

In most of the Michael addition reactions carried out during the synthesis of these amino-esters, the addition of a primary amine to methyl acrylate afforded a greater yield of the appropriate amino-ester than the corresponding addition to methyl methacrylate. In the mechanism previously postulated for the Michael addition of amines to

the double bond of methyl acrylate or methyl methacrylate, the presence of a β-methyl group in methyl methacrylate would be expected to offer increased steric resistance to the approach of a proton transfer agent compared to methyl acrylate. Since the concluding step is the rate-determining step, this will lead to a greater reactivity of methyl acrylate compared with methyl methacrylate.

An attempt to prepare 1,4-bis(2-methoxycarbonylethyl)-ethylenediamine (109) by mixing appropriate amounts of diethylamine and

$$H_3COOC(CH_2)_2NH(CH_2)_2NH(CH_2)_2COOCH_3$$
(109)

methyl acrylate in methanol led only to the isolation of a viscous oil. The synthesis of this compound had been previously reported (Brit. Patent 1966).

It has been shown by several workers that under certain conditions amides are the principle product of the reaction between amines and methyl acrylate or methyl methacrylate (Morsch 1933; Erickson 1952; Coutts 1971). During this present investigation the formation of amide by-products was observed during the synthesis of the various methyl 3-monoalkylaminopropionates. The amides, which were generally white solids, usually precipitated out when the methanol solvent was removed by rotary evaporation. The yields were always very small however, and no attempt was made to isolate any of these amides.

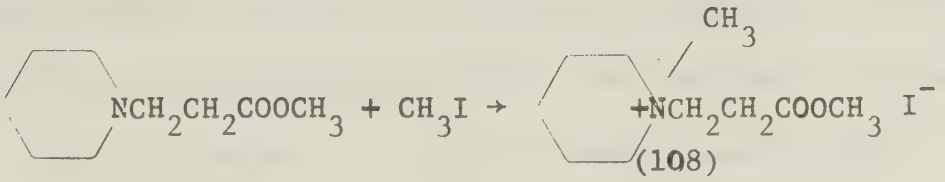
The methyl 3-monoalkylaminopropionates were colorless mobile

oils with a characteristic odor. When they were stored for some time at room temperature, colorless crystalline solids were slowly deposited in many cases. Coutts et al (1971) identified products formed in this way as 3-amino acids and suggested they were the result of hydrolysis by trace quantities of water. According to Bieber (1954) 2-methyl-3-piperidinopropionate on long standing in moist air or on shaking with cold water gave the free acid. The methyl 3-monoalkylaminopropionates synthesized here, even when stored in a desiccator, underwent hydrolysis to the corresponding 3-amino acids. No attempt was made to isolate or characterise any of the 3-amino acids so formed.

The hydrochloride salts of the methyl 3-monoalkylamino-propionates were prepared by adding diethyl ether saturated with dry hydrogen chloride to a stirred solution of the appropriate amino-ester base dissolved in ether. The precipitated hydrochlorides were purified by recrystallization and were found to be appreciably hygroscopic in some cases. These salts were not noticeably unstable; none of the melting points was accompanied by decomposition. On the other hand, with some of these compounds, the odor of acrylic esters was noticeable when a sample tube containing one of these salts was opened.

Several attempts to effect the methyl quaternization of methyl 2-methyl-3-piperidinopropionate were made using methyl iodide in different solvents such as methanol and acetone, all of which failed. It was concluded that this reaction was in some way sterically hindered, and it was therefore of interest to investigate quaternary salt formation in the corresponding straight chain compound methyl

3-piperidinopropionate (108) in order to establish if this hindrance to quaternization was in some way a property of the branched methyl group in the 2-position. When the free base and methyl iodide were mixed together the reaction mixture became very hot and the methiodide was isolated and characterized:



(108)

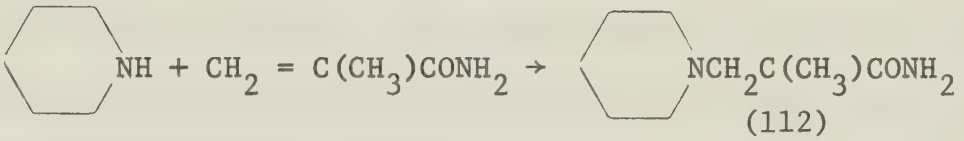
Apparently, the presence of branching in the 2-position was implicated in the resistance of 2-methyl-3-piperidinopropionate to quaternization. In view of this finding it was decided to investigate the quaternization of 2-methyl-3-dimethylaminopropionate. The pharmacology of the methiodide (111) has been reported by Schueler and Keasling (1951) but no reference to its synthesis could be found. Once again quaternization took place easily when the free base and methyl iodide were mixed together, the 2-methyl group offering no steric hindrance to quaternization.

Reference to Drieding stereomodels revealed that steric clashing between the branched methyl group in the 2-position and a quaternary methyl group in methyl 2-methyl-3-piperidinopropionate or one of the methyl groups on the quaternary nitrogen atom in methyl 2-methyl-3-dimethylaminopropionate methiodide was possible. Possibly, methyl 2-methyl-3-piperidinopropionate adopts a conformation such that approach to the lone pair of electrons on nitrogen by methyl iodide is

hindered by the 2-methyl group.

(ii) SYNTHESIS OF 2-METHYL-3-PIPERIDINOPROPIONAMIDE (112)

It was thought that it might be of interest to investigate amino amides structurally similar to certain amino-esters and amino-hydroxamic acids previously reported by Coutts et al (1969). In this regard 2-methyl-3-piperidinopropionamide (112) was prepared by a reaction analogous to that of amines with acrylic esters, namely, the addition of piperidine to methacrylamide:

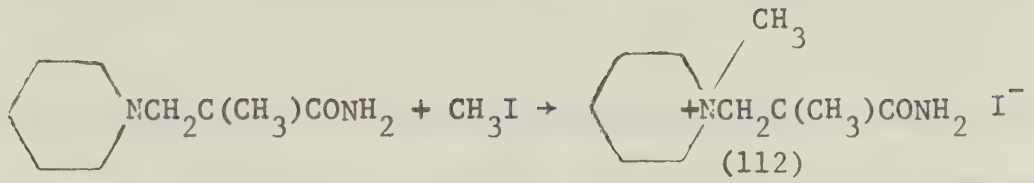


(112)

When the reactants were refluxed together in methanol, the addition of piperidine to methyl methacrylate afforded a good yield of 2-methyl-3-piperidinopropionamide (112). It was found that after storage at room temperature, 2-methyl-3-piperidinopropionamide began to show signs of decomposition; a persistent odor of piperidine was noticed over the distilled product. Erickson (1952) has commented on the decreased stability of 3-aminopropionamides when a 2-methyl group was present on the propionamide chain. This was shown by comparing the stability of N,N-dimethyl-3-dimethylaminopropionamide with N,N-dimethyl-2-methyl-3-dimethylaminopropionamide; the latter compound was the less stable.

The quaternary methiodide of 2-methyl-3-piperidinopropionamide (112) was synthesized quite easily by reacting the free base with methyl

iodide in acetone solution:



(112)

Methyl quaternization had a noticeable effect on the positions of certain absorption bands in the ir spectrum of 2-methyl-3-piperidinopropionamide. In the ir spectrum of a potassium bromide dispersion of the quaternary methiodide, the Amide I and Amide II bands shifted to higher wavenumbers relative to the free base· the elevation of the Amide II band was greater than that of the Amide I band. The νNH values of the free base both decreased on quaternization. The only explanation for this observation is that it is connected with differences in the crystal form of the free base and the methiodide. Nakanishi (1962) has pointed out that solid ir spectra of amides are especially sensitive to crystal orientation.

(iii) SYNTHESIS OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS

The 3-monoalkylaminopropionohydroxamic acids of general structure (48) prepared in this study were obtained by treating the appropriate methyl 3-monoalkylaminopropionate in anhydrous methanol with a solution of hydroxylamine hydrochloride in methanol (Coutts et al 1969; 1971):

$$RNHCH_2CHR^1COOCH_3 + NH_2OH \cdot HCl \rightarrow RN^+H_2CH_2CHR^1CONHOH\ Cl^- + CH_3OH$$

(48)

Reactions were run with the object of obtaining highly purified compounds, rather than high yields. The yields obtained, however, have been reported.

It was found that the 3-monoalkylaminopropionohydroxamic acid hydrochlorides prepared by this method were invariably contaminated by varying amounts of the hydrochloride salts of the 3-monoalkylaminopropionate precursors, even when the reaction times were prolonged. Accordingly, several recrystallizations were frequently required in order to obtain the amino hydroxamic acids in a pure state. Another difficulty encountered with some of these compounds was that they were often quite hygroscopic. It was therefore found necessary to employ anhydrous solvents during recrystallization.

The following 3-monoalkylaminopropionohydroxamic acids (48) were synthesized during this study:

3-Monoalkylaminopropionohydroxamic acid hydrochlorides

$$RN^{+}H_2CH_2CHR^1CONHOH\ Cl^{-}$$

(48)

Compound	R	R^1
113	CH_3	CH_3
114	CH_3CH_2	CH_3
115	$CH_3(CH_2)_2$	CH_3
116	$CH_3(CH_2)_3$	CH_3

Continued

3-Monoalkylaminopropionohydroxamic acid hydrochlorides (Continued)

Compound	R	R^1
117	$(CH_3)_3C$	H
118	$(CH_3)_3C$	CH_3
119	$CH_3(CH_2)_4$	CH_3
120		H
121		CH_3
122	$CH_3(CH_2)_5$	CH_3
123		H
124		CH_3
125	$CH_3(CH_2)_6$	CH_3
126	$CH_3(CH_2)_7$	H
128	$(CH_3)_3CCH_2C(CH_3)_2$	H
129	$(CH_3)_3CCH_2C(CH_3)_2$	CH_3
131	$CH_2 = CHCH_2$	CH_3

The 3-monoalkylaminopropionohydroxamic acid hydrochlorides were all colorless solids which melted with decomposition. Their ir spectra all showed a strong absorption band due to the stretching of the carbonyl group between 1630 and 1678 cm^{-1}.

Interestingly, the effect of a large bulky group on the amino

nitrogen atom in 3-cyclopentylaminopropionohydroxamic acid hydrochloride (120), 3-cyclohexylaminopropionohydroxamic acid hydrochloride (123) and 3-(1,1,3,3-tetramethylbutylamino)propionohydroxamic acid hydrochloride (128) was to lower the $\nu C = 0$ wavenumbers in comparison with the other compounds in the series. Conversely, the introduction of a branched methyl group on carbon 2 to give the corresponding branched chain compounds 2-methyl-3-cyclopentylaminopropionohydroxamic acid hydrochloride (121), 2-methyl-3-cyclohexylaminopropionohydroxamic acid hydrochloride (124) and 2-methyl-3-(1,1,3,3-tetramethylbutylamino)propionohydroxamic acid hydrochloride (129) shifted the $\nu C = 0$ values to a higher frequency.

The nmr characteristics of several of the 3-monoalkylaminopropionohydroxamic acid hydrochlorides were examined in DMSO-d_6. All displayed a 4-proton very broad signal in the range δ 8.13 - 11.12 ppm which collapsed after the addition of a drop of D_2O. This was attributed to the two protons of the NHOH group and the two protons of the N^+H_2 group. Coutts et al (1971) have examined acyclic hydroxamic acids of general structure (31) in DMSO-d_6. Three characteristic 1-proton broad signals within the δ 8.33 - 11.66 ppm range were observed, all of which exchanged in D_2O. One of these signals was located between δ 8.68 and 9.0 ppm and another between δ 10.05 and 10.58 ppm. These were due to the two protons of the NHOH group. The third signal was observed in all spectra between δ 10.75 and 11.05 ppm and was ascribed to the N^+H proton; this signal was absent when the spectra of bases rather than hydrochlorides were examined.

CHAPTER 4

PHARMACOLOGICAL EVALUATION OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS AND METHYL 3-MONOALKYLAMINOPROPIONATES

PHARMACOLOGICAL EVALUATION 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS AND METHYL 3-MONOALKYLAMINOPROPIONATES

(i) PRELIMINARY STUDIES ON THE BLOOD PRESSURE OF ANAESTHETIZED RATS

The effect of intravenous administration of a number of compounds on the blood pressure of anaesthetized rats was investigated. Each compound was tested at least twice in separate animals. The data obtained are shown in Tables 1, 2, and 3.

TABLE 1

EFFECT OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION MIN
113	1	3	-	-
	2	3	-	-
	4	4	-	-
114	1	1	-	-
	2	1	-	-
	4	2	-	-
115	1	2	-	-
	2	2	-	-
	4	2	-	-
116	1	2	-	-
	2	2	-	-
	4	2	-	-
117	1	2	-	-
	2	2	-	-
	4	3	-	-
118	1	2	-	-
	2	2	-	-
	4	2	-	-

Continued

TABLE 1 (Continued)

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION MIN
119	1	2	-	-
	2	2	-	-
	4	2	-	-
120	1	2	-	-
	2	2	-	-
	4	2	-	-
121	1	3	-	-
	2	3	-	-
	4	3	-	-
122	1	2	-	-
	2	2	-	-
	4	2	-	-
123	1	2	-	-
	2	2	-	-
	4	2	-	-
124	1	2	-	-
	2	2	-	-
	4	2	-	-
125	1	2	8	2
	2	2	14	3
	4	2	26	3
126	1	5	11	3
	2	5	17	4
	4	5	24	5
128	1	2	-	-
	2	2	-	-
	4	2	-	-
129	1	2	-	-
	2	2	-	-
	4	2	-	-
131	1	2	-	-
	2	2	-	-
	4	2	-	-

N.B. In the table, a dash denotes inactivity of a compound at the dose level shown.

TABLE 2

EFFECT OF METHYL 3-MONOALKYLAMINOPROPIONATE HYDROCHLORIDES ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION MIN
84	1	2	-	-
	2	2	-	-
	4	2	-	-
85	1	2	-	-
	2	2	-	-
	4	2	-	-
87	1	2	-	-
	2	2	-	-
	4	2	-	-
88	1	3	-	-
	2	3	-	-
	4	2	-	-
91	1	3	15	3
	2	3	13	7
	4	3	13	6
95	1	2	-	-
	2	2	-	-
	4	2	-	-
96	1	2	9	1
	2	2	11	1
	4	2	7	2
100	1	2	7	2
	2	2	10	3
	4	2	14	7
103	1	3	13	6
	2	2	26	7
	4	2	52	12
106	1	2	8	1
	2	2	11	1
	4	2	14	1

N.B. In the table, a dash denotes inactivity of a compound at the dose level shown.

TABLE 3

EFFECT OF 2-METHYL-3-PIPERIDINOPROPIONAMIDE METHIODIDE (112) ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

DOSE mg/kg	NUMBER OF DETERMINATIONS IN SEPARATE ANIMALS	AVERAGE PERCENTAGE FALL IN BLOOD PRESSURE	AVERAGE DURATION MIN
1	3	-	-
2	3	-	-
4	2	-	-

N.B. In the table, a dash denotes inactivity of the compound at the dose level shown.

(ii) EFFECT ON THE BLOOD PRESSURE OF HYOSCINE-TREATED RATS

To determine if the ability of the active compounds to lower blood pressure was due to a muscarinic action, the effect of intravenous administration of each drug on the blood pressure of at least two hyoscine-treated rats was determined. ACh (0.04 µg) was given, followed by a suitable dose of the compound under investigation. Hyoscine hydrobromide (1 mg) was then administered and the procedure repeated.

Only one compound, methyl 3-allylaminopropionate hydrochloride (106) displayed muscarinic activity. Four experiments were carried out and in each one the fall in blood pressure produced by amino-ester (106) was blocked by hyoscine.

The fall in blood pressure produced by 2-methyl-3-octylaminopropionate hydrochloride (103) (4 mg/kg) was not blocked in the presence of hyoscine, although hyoscine blocked the effects of ACh

completely. Two experiments were performed and one of the traces is shown in Fig. 1.

It was decided to carry out further experiments in order to gain more information concerning the mechanism of methyl 2-methyl-3-octylaminopropionate hydrochloride-induced hypotension.

(iii) EFFECT OF NICOTINE AND NOREPINEPHRINE ON THE BLOOD PRESSURE OF RATS TREATED WITH METHYL 2-METHYL-3-OCTYLAMINOPROPIONATE HYDROCHLORIDE (103)

Four experiments were performed in which the effects of nicotine and norepinephrine on the blood pressure of rats treated with methyl 2-methyl-3-octylaminopropionate hydrochloride (103) was investigated. A rise in the blood pressure of the rat was produced by giving nicotine (20 µg); three doses of norepinephrine (0.1, 0.2 and 0.4 µg) were then administered. The rats were injected with amino-ester (103) (4 mg/kg) and the procedure repeated. It was found in all four experiments that the nicotine-induced rise in blood pressure was completely blocked in the presence of amino-ester (103) while the pressor response to norepinephrine was only slightly lowered. Fig. 2 is representative of the results observed in all four experiments.

(iv) ISOLATED RABBIT INTESTINE EXPERIMENTS

In order to distinguish between ganglionic blocking activity and a possible adrenergic neurone blocking action in amino-ester (103), the isolated rabbit intestine preparation as described by Finkleman (1930) was used. This showed high spontaneous activity. Either stimulation of the sympathetic nerves, or the addition of norepinephrine,

caused the spontaneous contractions to cease and the whole muscle to relax. Conversely, the addition of either nicotine or ACh caused the muscle to contract.

In two experiments it was found that in the presence of a suitable concentration of amino-ester (103) the effect of nicotine was completely blocked whereas it had no effect on sympathetic nerve stimulation or on the action of norepinephrine. The amino-ester (103) was ineffective in preventing ACh-induced contractions of the isolated rabbit intestine. Fig. 3 shows one of the traces obtained.

(v) DETERMINATION OF THE EFFECTIVENESS OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES IN PROTECTING MICE AGAINST LETHAL ORGANOPHOSPHATE POISONING

The effectiveness of various 3-monoalkylaminopropionohydroxamic acid hydrochlorides, given sc, in protecting against a 5 mg/kg dose of DFP, given ip, in male Alas strain mice was investigated.

The LD_{50} of the DFP in these mice was determined from the results shown in Table 4 obtained by Dr. D.F. Biggs of the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta.

TABLE 4

DETERMINATION OF THE LD_{50} OF DFP IN MALE ALAS STRAIN MICE

DOSE mg/kg	NUMBER DEAD	NUMBER ALIVE
2.0	2	8
2.5	6	4
3.0	9	1

The doses given (mg/kg) were plotted against percentage mortality on logarithmic probability paper and the upper and lower confidence limits for 19/20 probability determined by the method of Litchfield and Wilcoxon (1949). The LD_{50} was found to be 2.38 (2.09 to 2.71) mg/kg.

Each compound was screened once at a dose of 200 mg/kg in groups of three mice. The results obtained are shown in Table 5.

TABLE 5

EFFECTIVENESS OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES[a] AGAINST DFP[b] TOXICITY IN MICE

COMPOUND NUMBER IN TEXT	NUMBER DEAD	NUMBER ALIVE
113	3	-
114	3	-
115	3	-
116	3	-
117	3	-
118	3	-
119	2	1
120	3	-
121	3	-
122	3	-
123	3	-
124	3	-
125	3	-
126	3	-
127	2	1
128	3	-
129	3	-
Control[c]	3	-

[a] Given sc (200 mg/kg) 30 minutes before the DFP.

[b] Given ip (5 mg/kg).

[c] 0.2 ml of distilled water.

The mice generally died within five minutes; a few succumbed after one hour and the remaining two animals were still alive after one day's observation. These survivors had been injected with 2-methyl-3-pentylaminopropionohydroxamic acid hydrochloride (119) and 3-(1,1,3,3-tetramethylbutylamino)propionohydroxamic acid hydrochloride (127) respectively. It was decided to investigate these two compounds further using larger doses. The results are shown in Table 6.

TABLE 6

EFFECTIVENESS OF 2-METHYL-3-PENTYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDE (119)[a] AND 3-(1,1,3,3-TETRAMETHYLBUTYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDE (127)[a] AGAINST DFP[b] TOXICITY IN MICE

COMPOUND NUMBER IN TEXT	DOSE mg/kg	NUMBER DEAD	NUMBER ALIVE
119	200	3	-
	400	3	-
127	200	1	2
	400	1	2
Control[c]		3	-

[a] Given sc 30 minutes before the DFP.

[b] Given ip (5 mg/kg).

[c] 0.2 ml of distilled water.

These results suggested that 3-(1,1,3,3-tetramethylbutylamino)propionohydroxamic acid hydrochloride (127) was effective in protecting mice against the toxic effects of DFP. Accordingly, further studies were undertaken in which various doses of this compound were

administered to mice followed by a 5 mg/kg dose of DFP. Table 7 shows the data obtained.

TABLE 7

EFFECTIVENESS OF VARIOUS DOSES OF 3-(1,1,3,3-TETRAMETHYLBUTYLAMINO)-PROPIONOHYDROXAMIC ACID HYDROCHLORIDE (127)[a] AGAINST DFP[b] TOXICITY IN MICE

DOSE (mg/kg)	NUMBER DEAD	NUMBER ALIVE
50	9	1
100	10	-
200	10	-
400	9	1
Control[c]	9	1

[a] Given sc 20 minutes before the DFP.

[b] Given ip (5 mg/kg).

[c] 0.2 ml of distilled water.

Contrary to the earlier results 3-(1,1,3,3-tetramethylbutylamino)-propionohydroxamic acid hydrochloride was ineffective in protecting mice from the lethal effects of DFP.

CHAPTER 5

DISCUSSION OF THE PHARMACOLOGY OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS AND METHYL 3-MONOALKYLAMINOPROPIONATES

DISCUSSION OF THE PHARMACOLOGY OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS AND METHYL 3-MONOALKYLAMINOPROPIONATES

(i) EFFECT OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACIDS ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

The pharmacological results obtained for the 3-monoalkylaminopropionohydroxamic acids evaluated are given in Table 1. Each structural type will now be briefly discussed.

The following 3-monoalkylaminopropionohydroxamic acids of general structure (48) where R was a straight alkyl chain were examined:

i) 3-Monoalkylaminopropionohydroxamic acids

$$RN^{+}H_2CHR^{1}CONHOH\ Cl^{-}$$

(48)

Compound	R	R^1
113	CH_3	CH_3
114	CH_3CH_2	CH_3
115	$CH_3(CH_2)_2$	CH_3
116	$CH_3(CH_2)_3$	CH_3
117	$CH_3(CH_2)_4$	CH_3
121	$CH_3(CH_2)_5$	CH_3
125	$CH_3(CH_2)_6$	CH_3
126	$CH_3(CH_2)_7$	H
131	$CH_2 = CHCH_2$	CH_3

Coutts' group (1971) studied several of the corresponding compounds of general structure (48) where R^1 was a hydrogen atom:

3-Monoalkylaminopropionohydroxamic acids (Coutts et al 1971)

$$RN^{+}H_2CHR^{1}CONHOH\ Cl^{-}$$
(48)

Compound	R	R^1
49	CH_3	H
50	CH_3CH_2	H
51	$CH_3(CH_2)_2$	H
53	$CH_3(CH_2)_3$	H
54	$CH_3(CH_2)_5$	H

These investigators found that with the exception of 3-methylpropionohydroxamic acid hydrochloride (49), which caused a rise, all of these compounds produced a fall in blood pressure of the anaesthetized cat.

The introduction of a methyl group on carbon 2 in 3-ethylaminopropionohydroxamic acid hydrochloride (50), 3-propylaminopropionohydroxamic acid hydrochloride (51), 3-butylaminopropionohydroxamic acid hydrochloride (53) and 3-hexylaminopropionohydroxamic acid hydrochloride (54) to give the corresponding branched chain compounds (114, 115, 116 and 122) resulted in a complete loss of hypotensive activity. 2-Methyl-3-propylaminopropionohydroxamic acid hydrochloride (115) was found by Coutts et al (1971) to cause a fall in blood pressure of the anaesthetized cat, although both the intensity and duration of this fall were less than that of its straight chain analogue 3-propylaminopropionohydroxamic acid hydrochloride (51). This compound, however, was

inactive in the anaesthetized rat, a fact in accord with the observed inactivity of other lower 2-methyl-3-monoalkylaminopropionohydroxamic acid hydrochlorides (114, 116 and 119).

The addition of a methyl group in the 2-position to 3-methylaminopropionohydroxamic acid hydrochloride (49) giving the branched analogue (113) abolished the pressor activity observed by Coutts and his co-workers in the straight chain compound.

2-Methyl-3-pentylaminopropionohydroxamic acid hydrochloride (119) was inactive, but since the unbranched analogue was not to hand, no conclusions could be drawn regarding the possible inactivating role of the 2-methyl group in this compound.

In 2-methyl-3-heptylaminopropionohydroxamic acid hydrochloride (125) a significant change was observed in the inactivating effect of a methyl group in the 2-position. Table 1 shows that this compound caused a fall in blood pressure which was dose dependent. Apparently, when the alkyl chain of the amine part of the molecule is longer than n-hexyl, a methyl group in the 2-position no longer completely inactivates the molecule.

3-Octylaminopropionohydroxamic acid hydrochloride (126) also produced a fall in blood pressure in the anaesthetized rat which was not dose dependent (Table 1). Unfortunately, the branched analogue 2-methyl-3-octylaminopropionohydroxamic acid hydrochloride (127) could not be prepared so that the effect of having a 2-methyl function in the molecule could not be tested.

The unsaturated compound 2-methyl-3-allylaminopropionohydroxamic

acid hydrochloride (131), like its saturated analogue 2-methyl-3-propylaminopropionohydroxamic acid hydrochloride (115) was inactive. The straight chain compound 3-allylaminopropionohydroxamic acid hydrochloride (130) could not be crystallized so this compound could not be compared with its saturated analogue 3-propylaminopropionohydroxamic acid hydrochloride (51) (Coutts et al 1971).

It was decided to study further examples of 3-monoalkylaminopropionohydroxamic acids of general structure (48) to extend knowledge of general structure-activity relationships of these compounds. The following compounds of general structure (48) where R was a highly branched group were evaluated pharmacologically:

ii) 3-Monoalkylaminopropionohydroxamic acids

(48)

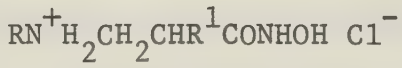
Compound	R	R^1
117	$(CH_3)_3C$	H
118	$(CH_3)_3C$	CH_3
128	$(CH_3)_3CCH_2C(CH_3)_2$	H
131	$(CH_3)_3CCH_2C(CH_3)_2$	CH_3

3-tert-Butylaminopropionohydroxamic acid hydrochloride (117), 3-(1,1,-3,3-tetramethylbutylamino)propionohydroxamic acid hydrochloride (128) and the branched analogues (118) and (131) were all inactive. Hence,

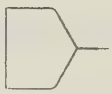
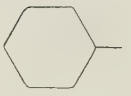
presence of a bulky highly branched group attached to the amino nitrogen atom was not conducive to hypotensive activity.

Attention was then focused on 3-monoalkylaminopropionohydroxamic acids (48) where the group R was cyclic and the following compounds were studied:

iii) 3-Monoalkylaminopropionohydroxamic acids



(48)

Compound	R	R^1
120		H
121		CH_3
123		H
124		CH_3

The pharmacological data obtained are given in Table 1; 3-cyclopentylaminopropionohydroxamic acid hydrochloride (120), 3-cyclohexylaminopropionohydroxamic acid hydrochloride (123) and the branched chain

analogues (121) and (124) all proved inactive.

The most important deduction to emerge from the pharmacological data is that in aminohydroxamic acids of general structure (48) the nature of the group R is highly critical. The introduction of a methyl group R^1 can inactivate the molecule, depending on the chain length of the group R. In compounds where R is a straight chain and R^1 is a hydrogen atom, in the absence of any data concerning those compounds where R is n-pentyl or n-heptyl, n-hexyl appears to be the optimum chain length necessary for significant hypotensive activity (Coutts et al 1971).

(ii) EFFECT OF METHYL 3-MONOALKYLAMINOPROPIONATES ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

The pharmacological data for the methyl 3-monoalkylaminopropionates are given in Table 2.

The following methyl 3-monoalkylaminopropionates of general structure (55) where R was an unbranched alkyl group were investigated:

i) Methyl 3-monoalkylaminopropionates

$$RN^+H_2CH_2CHR^1COOCH_3\ Cl^-$$
(55)

Compound	R	R^1
91	$CH_3(CH_2)_4$	H
101	$CH_3(CH_2)_6$	H
103	$CH_3(CH_2)_7$	CH_3
106	$CH_2 = CHCH_2$	H

Methyl 3-pentylaminopropionate hydrochloride (91) was found to lower the blood pressure (Table 2); this blood pressure lowering was not dose dependent. Methyl 3-heptylaminopropionate hydrochloride (101) also caused a fall in blood pressure which was not dose dependent.

The most active compound in causing a fall in blood pressure of any of the compounds examined was methyl 2-methyl-3-octylamino-propionate hydrochloride (103). The relevant data in Table 5 shows this fall in blood pressure was dose dependent. The compound produced a maximum fall in blood pressure immediately after injection.

The possibility of amino-ester (103) having muscarinic activity was ruled out because the fall in blood pressure produced by this compound in the anaesthetized rat was not blocked by hyoscine, whereas that produced by ACh was (Fig. 1). However, it did block a nicotine-induced rise in blood pressure while the pressor response to norepinephrine was only slightly lowered (Fig. 2). These observations suggested two possible mechanisms of action for methyl 2-methyl-3-octyl-aminopropionate hydrochloride; either a ganglion blocking action or an adrenergic neurone blocking action. A consideration of structure-activity relationships amongst adrenergic neurone blocking drugs suggested that a ganglionic blocking action was the more likely. The slight lowering of the pressor response to norepinephrine indicated that amino-ester (103) might also possess extremely weak sympatholytic activity. This was not sufficient, however, to account for the fall in blood pressure produced by this compound.

The suspicion that amino-ester (103) might be causing a fall

in blood pressure by ganglionic blockade rather than adrenergic neurone blockade was confirmed by the results obtained in isolated rabbit intestine experiments (Fig. 3). It completely blocked the stimulant action of nicotine on the parasympathetic ganglia, while the responses of the gut to sympathetic nerve stimulation and norepinephrine were unaffected. There was no effect on the response of the gut to ACh, ruling out any possibility of amino-ester (103) antagonising the action of ACh at the postganglionic cholinergic receptor.

These results indicate that 2-methyl-3-octylaminopropionate hydrochloride (103) blocks sympathetic and parasympathetic ganglia.

Methyl 3-allylaminopropionate hydrochloride (106) was found to possess weak muscarinic activity. It can be regarded as being derived from the reversed carboxyl analogue of ACh by replacement of two of the methyl groups attached to the onium atom by hydrogen atoms, and replacement of the third methyl group by an allyl group. The reversed carboxyl analogue of ACh has been shown to possess appreciable muscarinic activity (Bass et al 1950; Barrass et al 1968) so the muscarinic activity observed in methyl 3-allylaminopropionate hydrochloride is not unexpected.

A comparison of methyl 3-allylaminopropionate hydrochloride (106) with its saturated analogue methyl 3-propylaminopropionate hydrochloride (58) is interesting.

$$CH_2 = CHCH_2N^+H_2CH_2CH_2COOCH_3\ Cl^-$$
(106)

$$CH_3CH_2CH_2N^+H_2CH_2CH_2COOCH_3\ Cl^-$$
(58)

The latter compound has been shown to cause a rise in blood pressure in the anaesthetized cat (Coutts et al 1971). The substitution of an allyl group for a propyl group in amino-ester (58) appears to profoundly affect the activity of the molecule.

Several amino-esters of general structure (55) where the alkyl chain R was branched were also studied:

ii) Methyl 3-monoalkylaminopropionates

$$RN^+HCH_2CHR^1COOCH_3\ Cl^-$$
(55)

Compound	R	R^1
84	$(CH_3)_2CH$	H
85	$(CH_3)_2CH$	CH_3
87	$CH_3CH_2CHCH_3$	H
88	$CH_3CH_2CHCH_3$	CH_3
95	$(CH_3)_2CH(CH_2)_2$	H
96	$(CH_3)_2CH(CH_2)_2$	CH_3

Table 2 shows that one compound, 2-methyl-3-isopentylaminopropionate hydrochloride (96) had weak hypotensive activity; the rest were inactive.

Several related compounds of general structure (55) where

R was an unbranched alkyl chain were evaluated by Coutts et al (1971):

Methyl 3-monoalkylaminopropionates (Coutts et al 1971)

$$RN^{+}H_2CH_2CHR^{1}COOCH_3\ Cl^{-}$$

(55)

Compound	R	R^1
57	CH_3CH_2	H
58	$CH_3(CH_2)_2$	H
60	$CH_3(CH_2)_3$	H

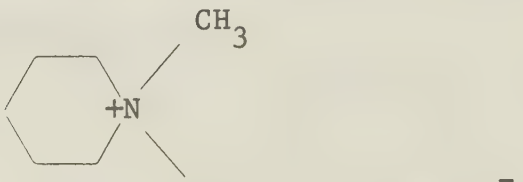
All these amino-esters possessed hypotensive activity, whereas the corresponding compounds with the branched methyl group on the alkyl chain R, methyl 3-isopropylaminopropionate hydrochloride (84), methyl 3-sec-butylaminopropionate hydrochloride (87) and methyl 3-isopentylaminopropionate hydrochloride (95) were inactive. Evidently, the introduction of a branched methyl group onto the alkyl chain R inactivates the molecule. The actual position of this methyl group does not appear to be important; in amino-ester (87) it was adjacent to the amino nitrogen atom; in amino-ester (95) it was adjacent to the terminal carbon atom of the alkyl chain.

2-Methyl-3-isopropylaminopropionate hydrochloride (107) and 2-methyl-3-sec-butylaminopropionate hydrochloride (110) were both inactive. Interestingly, 2-methyl-3-isopentylaminopropionate

hydrochloride (95) was active. The analogous straight chain compound methyl 3-butylaminopropionate hydrochloride (60) possessed hypotensive activity, but the compound with a branched methyl group on the alkyl chain R, methyl 3-isopentylaminopropionate hydrochloride (95) did not. When a branched methyl group was introduced into the 2-position, however, the molecule once again possessed hypotensive activity.

(iii) EFFECT OF 2-METHYL-3-PIPERIDINOPROPIONAMIDE METHIODIDE (112) ON ARTERIAL BLOOD PRESSURE OF THE ANAESTHETIZED RAT

Table 3 shows that 2-methyl-3-piperidinopropionamide methiodide (121) had no affect on the blood pressure of the anaesthetized rat.



(112)

Barrass' group (1968) have studied several related amino amides of general structure (28) where R was a pyrrolidino or dimethylamino group and R^1 was a methyl group or a hydrogen atom. The quaternary salts had

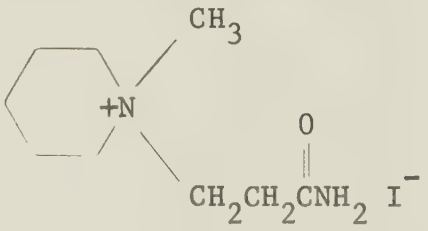
$$R(CH_2)_nCONR^1_2 \quad n = 1 \text{ to } 4$$

(28)

nicotinic activity. A comparison of molecular models of these compounds with those of standard nicotinic agents showed that in every case, a quaternary nitrogen atom was separated from a carbonyl oxygen atom by

4 - 4.5Å. As a working hypothesis, Barrass and his co-workers assumed that the nicotinic receptor had at least two essential sites separated by 4 - 4.5Å, although all the known facts could not be accommodated by such a receptor model.

A Drieding stereomodel of 2-methyl-3-piperidinopropionamide methiodide (112) showed that the quaternary nitrogen atom was separated from the carbonyl oxygen atom by 4.5Å and could thus fit the proposed sites on the receptor. The lack of nicotinic activity is probably due to the branched methyl group in the 2-position interfering with binding at one or the other of the two active sites. Unfortunately, lack of time prevented the synthesis of the straight chain compound 3-piperidinopropionamide methiodide (132).



(132)

(iv) EFFECTIVENESS OF 3-MONOALKYLAMINOPROPIONOHYDROXAMIC ACID HYDROCHLORIDES IN PROTECTING AGAINST LETHAL ORGANOPHOSPHATE POISONING

The ability of the 3-monoalkylaminopropionohydroxamic acid hydrochlorides to protect mice against lethal poisoning by DFP was investigated. Tables 5, 6 and 7 show the results obtained. None of these compounds, when given sc, was considered effective in protecting against DFP poisoning.

CHAPTER 6

EXPERIMENTAL CHEMISTRY

NOTES ON THE EXPERIMENTAL DATA

Melting points were determined on a Thomas Hoover capillary melting point apparatus. All melting points and boiling points are uncorrected. Ir spectra were recorded on a Beckman Infrared Spectrophotometer Model 10. Nmr spectra were taken on a Varian Associates Model A-60D spectrometer. When the solvent employed was D_2O, DSS was used as the standard; in other solvents the reference was TMS. Elemental analyses were performed by the Faculty of Pharmacy and Pharmaceutical Sciences and the Microanalytical Laboratory, Department of Chemistry, University of Alberta. All the commercial chemicals were used without further purification.

SYNTHESIS OF AMINO-ESTERS AND RELATED COMPOUNDS

General Preparative Methods

Methyl acrylate or methyl methacrylate was added to an equimolecular quantity of the appropriate amine under the conditions below. During the reaction period the flask was either kept in the dark or covered with foil to prevent light-catalyzed polymerization of the methyl acrylate or methyl methacrylate reactant, no polymerization inhibitor being employed. The reaction was judged complete by the absence of C = C stretching vibrations near 1630 and 1640 cm^{-1} respectively in the ir spectrum of the crude product (due to the acrylate or methacrylate starting materials). The esters were then isolated by fractional distillation under reduced pressure and collected as colorless mobile oils.

Method 1 (Ziering et al 1947)

The amine (0.25 mol) was dissolved in anhydrous methanol (100 ml) and the solution cooled in an ice-salt mixture. Ice-cold methyl acrylate or methyl methacrylate (0.25 mol) was added to the stirred amine solution in small aliquots. The resulting mixture was then allowed to stand at room temperature from 12 hr. to 7 weeks. The methanol was removed under vacuum on a rotary evaporator and the resulting oil distilled through a Vigreux column and the appropriate fraction collected.

Method 2

Methyl acrylate or methyl methacrylate (0.25 mol) was added to a solution of the appropriate amine (0.25 mol) in anhydrous methanol (50 - 100 ml). The reaction mixture was then heated under reflux for 20 hr. to 7 days. The solvent was removed by rotary evaporation and the residual oil fractionally distilled under reduced pressure.

Preparation of the Hydrochloride Salts of the Amino-Esters

The hydrochloride salts of the esters were obtained by adding anhydrous diethyl ether saturated with dry hydrogen chloride to a stirred ether solution of the appropriate ester cooled in ice-salt. The precipitated hydrochlorides were recrystallized from methanol-ether unless specified otherwise. They were obtained as colorless solids which were characterized by their infrared spectra. All the compounds examined showed N^{+}-H stretching bands in the 2360 - 2790 cm^{-1} region (Heacock and Marion 1956).

Methyl 2-methyl-3-methylaminopropionate (81)

Methyl methacrylate (75.1g; 0.75 mol) in anhydrous methanol (100 ml) was added with stirring to a cooled solution of methylamine (15.53g; 0.5 mol) in absolute ethanol (50 ml) over a period of 90 minutes. The product, after standing for 5 days at room temperature, was fractionally distilled under reduced pressure to give the title compound (13.15g; 34%) as a colorless oil bp 74 - 75° (26 mm). Reported (Howton 1945) bp 48.8 - 49.5 (8 mm); (Horii et al 1962) bp 48 - 50° (9 mm).

Ir spectrum (film): 3360 (N-H), 1731 (C = 0) cm^{-1}.

Anal. Found: C, 55.29; H, 10.03. $C_6H_{13}NO_2$ requires: C, 54.91; H, 9.99.

The hydrochloride was recrystallized from acetone-ether; mp 80°.

Methyl 2-methyl-3-ethylaminopropionate (82)

Using general method 1 for the synthesis of amino-esters, the title compound was prepared in 47% yield from ethylamine and methyl methacrylate. The reaction mixture was allowed to stand at room temperature for 3 days. The product had bp 76° (18.5 mm). Reported (David and Sinay 1965) bp 51° (0.5 mm).

Ir spectrum (film): 3338 (N-H), 1739 (C = 0) cm^{-1}.

The hydrochloride salt of the title compound melted at 104 - 105°.

Anal. Found: C, 46.30; H, 8.57. $C_7H_{16}ClNO_2$ requires: C, 46.30; H, 8.88.

Methyl 2-methyl-3-n-propylaminopropionate (83)

When general method 1 for the synthesis of amino-esters was employed the title compound was obtained from n-propylamine and methyl methacrylate in 54% yield. The reaction mixture was kept at room temperature for 21 hr. The title compound had bp 88 - 89° (17.5 mm). Reported (Pfau 1967) bp 31° (0.15 mm).

Ir spectrum (film): 3329 (N-H), 1740 (C = O) cm^{-1}.

Anal. Found: C, 60.58; H, 10.99. $C_8H_{17}NO_2$ requires: C, 60.30; H, 10.76.

The mp of the hydrochloride was 127 - 128°.

Methyl 3-isopropylaminopropionate (84)

Using general method 1 for the synthesis of amino-esters, the title compound was obtained from isopropylamine and methyl acrylate in 67% yield. The reaction mixture was left standing at room temperature for 6 days. The title compound had bp 80 - 82° (24.5 mm).

Ir spectrum (film): 3336 (N-H), 1742 (C = O) cm^{-1}.

Recrystallization of the hydrochloride from acetone gave needles, mp 107°.

Anal. Found: C, 46.66; H, 9.44. $C_7H_{16}ClNO_2$ requires: C, 46.30; H, 9.30.

Methyl 2-methyl-3-isopropylaminopropionate (85)

The title compound was prepared from isopropylamine and methyl methacrylate in 44% yield by the general method 1 for the synthesis of amino-esters. The reactants were kept at room temperature for 5 days. The title compound had bp 83 - 84° (22.5 mm). Reported (Smith and

Binkley 1959) bp 48° (2 mm); (Pfau 1967) bp 41° (0.1 mm).

Ir spectrum (film): 3334 (N-H); 1739 (C = O) cm^{-1}.

Its hydrochloride had mp 111 - 112°. Reported (Pfau 1967) mp 114 - 114.5°.

Anal. Found: C, 48.61; H, 9.22. $C_8H_{18}ClNO_2$ requires: C, 49.10; H, 9.27.

Methyl 2-methyl-3-n-butylaminopropionate (86)

The title compound was obtained in 40% yield from n-butylamine and methyl methacrylate using general method 1 for the synthesis of amino-esters. The reaction mixture was kept for 16 hr. at room temperature. The product had bp 104° (19 mm). Reported (Luskin et al 1956) bp 58 - 60° (1 mm); (Biniecki and Gutkowska, 1966) 89 - 90° (8 mm).

Ir spectrum (film): 3333 (N-H); 1740 (C = O) cm^{-1}.

Anal. Found: C, 62.26; H, 11.24. $C_9H_{19}NO_2$ requires: C, 62.36; H, 11.06.

The hydrochloride had mp 130 - 131°.

Methyl 3-sec-butylaminopropionate (87)

Using general method 1 for the synthesis of amino-esters, the title compound was obtained in 38% yield from sec-butylamine and methyl acrylate. The reactants were stood at room temperature for 7 weeks. The title compound had bp 95 - 97° (22.5 mm).

Ir spectrum (film): 3338 (N-H); 1741 (C = O) cm^{-1}.

The hydrochloride was recrystallized from acetone-ether giving needles, mp 71.5 - 72.5°.

Anal. Found: C, 48.89; H, 9.56. $C_8H_{18}ClNO_2$ requires: C, 49.10; H, 9.27.

Methyl 2-methyl-3-sec-butylaminopropionate (88)

When general method 1 for the synthesis of amino-esters was employed, the title compound was obtained in 39% yield from sec-butylamine and methyl methacrylate. The reaction mixture was left at room temperature for 7 weeks. The product had bp 99° (22.5 mm).

Ir spectrum (film): 3343 (N-H); 1741 (C = O) cm^{-1}.

Recrystallization of the hydrochloride afforded needles, mp 75 - 78°.

Anal.Found: C, 51.50; H, 9.54. $C_9H_{20}ClNO_2$ requires: C, 51.55; H, 9.61.

Methyl 3-tert-butylaminopropionate (89)

The title compound was prepared in 56% yield from tert-butylamine and methyl acrylate using general method 1 for the synthesis of amino-esters. The reactants were kept at room temperature for 6 weeks. The title compound had bp 89 - 90° (24 mm).

Ir spectrum (film): 3325 (N-H); 1737 (C = O) cm^{-1}.

The hydrochloride was recrystallized to give needles, mp 142°.

Nmr spectrum (D_2O): δ 3.73 (3-proton singlet, OCH_3); 2.67 - 3.50 (4-proton complex multiplet, $C\underline{H}_2C\underline{H}_2$); 1.38 ppm (9-proton singlet, $(CH_3)_3C$).

Anal. Found: C, 49.31; H, 9.53. $C_8H_{18}ClNO_2$ requires: C, 49.10; H, 9.27.

Methyl 2-methyl-3-tert-butylaminopropionate (90)

Using general method 1 for the synthesis of amino-esters, the title compound was prepared from tert-butylamine and methyl methacrylate in 42% yield. The reaction mixture was kept for 6 weeks at room temperature. The compound had bp 89 - 90° (24 mm). Reported(Luskin et al 1956) bp 49 - 50° (1 mm).

Ir spectrum (film): 3330 (N-H); 1740 (C = O) cm^{-1}.

The mp of the hydrochloride was 142°.

Nmr spectrum (D_2O): δ 3.73 (3-proton singlet, OCH_3); 2.81 - 3.38 (3-proton complex multiplet, CH_2CH); 1.78 (singlet, $(CH_3)_3C$); 1.21 (doublet, J = 7 Hz, CH_3); combined signals at 1.78 and 1.21 ppm integrate for 12 protons.

Anal. Found: C, 49.31; H, 9.53. $C_9H_{20}ClNO_2$ requires: C, 49.10; H, 9.27.

Methyl 3-n-pentylaminopropionate (91)

The title compound was obtained in 51% yield from n-pentylamine and methyl acrylate using general method 2 for the synthesis of amino-esters. The reaction mixture was heated under reflux for 24 hr. The product had bp 115° (22 mm).

Ir spectrum (film): 3331 (N-H); 1743 (C = O) cm^{-1}.

Anal. Found: C, 62.05; H, 10.80. $C_9H_{19}NO_2$ requires: C, 62.36; H, 11.06.

Hydrochloride salt melted at 195.5°.

Methyl 2-methyl-3-n-pentylaminopropionate (92)

When general method 2 for the synthesis of amino-esters was employed and the reactants were heated under reflux for 24 hr., the title compound was prepared in 58% yield from n-pentylamine and methyl methacrylate. It had bp 120° (22.5 mm).

Ir spectrum (film): 3344 (N-H); 1742 (C = 0) cm^{-1}.

Anal. Found: C, 64.23; H, 11.23. $C_{10}H_{21}NO_2$ requires: C, 64.10; H, 11.31.

The hydrochloride was recrystallized from acetone-ether; mp 140°.

Methyl 3-cyclopentylaminopropionate (93)

The title compound was prepared from cyclopentylamine and methyl acrylate in 38% yield using general method 1 for the synthesis of amino-esters. The reaction mixture was kept at room temperature for 27 hr. The product had bp 119 - 121° (20 mm).

Ir spectrum (film): 3332 (N-H); 1740 (C = 0) cm^{-1}.

The hydrochloride was recrystallized from acetone to give needles, mp 104 - 105°. It was recrystallized (acetone-ether) without a change in the melting point.

Anal. Found: C, 51.74; H, 9.06. $C_9H_{18}ClNO_2$ requires: C, 51.98; H, 8.73.

Methyl 2-methyl-3-cyclopentylaminopropionate (94)

Using general method 1 for the synthesis of amino-esters, the title compound was obtained in 22% yield from cyclopentylamine and methyl methacrylate. The reaction mixture was kept at room temperature for

27 hr. The title compound had bp 122 - 123° (20.5 mm).

Ir spectrum (film): 3331 (N-H); 1736 (C = O) cm^{-1}.

The hydrochloride salt was recrystallized from acetone-ether; mp 85 - 86°.

Anal. Found: C, 54.26; H, 9.20. $C_{10}H_{20}ClNO_2$ requires: C, 54.16; H, 9.09.

Methyl 3-isopentylaminopropionate (95)

The title compound was obtained in 34% yield from isopentylamine and methyl acrylate when general method 2 for the synthesis of amino-esters was used. The reaction products were boiled under reflux for 23 hr. The title compound had bp 111 - 113° (21 mm).

Ir spectrum (film): 3345 (N-H); 1741 (C = O) cm^{-1}.

Anal. Found: C, 62.48; H, 10.89. $C_9H_{19}NO_2$ requires: C, 62.36; H, 11.06.

The hydrochloride of the title compound had mp 208°.

Methyl 2-methyl-3-isopentylaminopropionate (96)

When general method 2 for the synthesis of amino-esters was employed and the isopentylamine and methyl methacrylate reactants were heated under reflux for 23 hr., the title compound was prepared in 46% yield. It had bp 116 - 117° (22.5 mm).

Ir spectrum (film): 3339 (N-H); 1741 (C = O) cm^{-1}.

Anal. Found: C, 64.31; H, 11.10. $C_{10}H_{21}NO_2$ requires: C, 64.10; H, 11.31.

The hydrochloride salt of the title compound was recrystallized from methanol-ether. It was then recrystallized from acetone.

The hydrochloride sintered at 110° and melted at 119°. It was recrystallized (acetone) without a change in the melting point.

Methyl 2-methyl-3-n-hexylaminopropionate (97)

Using general method 2 for the synthesis of amino-esters, the title compound was obtained from n-hexylamine and methyl methacrylate in 37% yield. The reaction mixture was heated under reflux for 30 hr. The title product had bp 133 - 134° (21 mm).

Ir spectrum (film): 3341 (N-H); 1738 (C = O) cm^{-1}.

Anal. Found: C, 65.81. H, 11.54. $C_{11}H_{23}NO_2$ requires: C, 65.61; H, 11.52.

The hydrochloride had mp 126 - 127°.

Methyl 3-cyclohexylaminopropionate (98)

When general method 2 for the synthesis of amino-esters was used, the title compound was obtained in 63% yield from cyclohexylamine and methyl acrylate. The reaction mixture was left at room temperature for 12 hr. The title compound had the bp 132 - 133° (17.5 mm). Reported (Southwick and Crouch 1953) bp 125 - 128° (4.5 mm).

Ir spectrum (film): 3320 (N-H); 1734 (C = O) cm^{-1}.

The hydrochloride was recrystallized several times to give material of analytical purity, mp 161 - 162°. Reported (Southwick and Crouch 1953) 163 - 164°.

Anal. Found: C, 54.11; H, 9.12; N, 6.38. $C_{10}H_{20}ClNO_2$ requires: C, 54.16; H, 9.09; N, 6.32.

Methyl 2-methyl-3-cyclohexylaminopropionate (99)

The title compound was obtained from cyclohexylamine and methyl

methacrylate in 42% yield by the general method 1 for the synthesis of amino-esters. The reactants were kept at room temperature for 3 days. The title product had bp 141° (22 mm). Reported (Horii et al 1962) 110° (7 mm).

Ir spectrum (film): 3328 (N-H); 1739 (C = O) cm^{-1}.

Its hydrochloride had mp 157°. Reported (Horii et al 1962) 156°.

Anal. Found: C, 56.12; H, 9.47. $C_{11}H_{22}ClNO_2$ requires: C, 56.04; H, 9.41.

Methyl 3-n-heptylaminopropionate (100)

The title compound was prepared in 45% yield from n-heptylamine and methyl acrylate using general method 2 for the synthesis of amino-esters. The reactants were heated under reflux for 15 hr. The title compound had bp 86 - 89° (0.06 mm).

Ir spectrum (film): 3338 (N-H); 1742 (C = O) cm^{-1}.

Anal. Found: C, 65.27; H, 11.42. $C_{11}H_{23}NO_2$ requires: C, 65.61; H, 11.52.

The hydrochloride salt melted at 181 - 182°.

Methyl 2-methyl-3-n-heptylaminopropionate (101)

Using general method 2 for the synthesis of amino-esters, the title compound was prepared in 52% yield from n-heptylamine and methyl methacrylate after heating under reflux for 37 hr. The title compound had the bp 82 - 84° (0.03 mm).

Ir spectrum (film): 3342 (N-H); 1743 (C = O) cm^{-1}.

Anal. Found: C, 66.93; H, 11.40. $C_{12}H_{25}NO_2$ requires: C, 66.93; H, 11.71.

The hydrochloride salt had mp 115°.

Methyl 3-n-octylaminopropionate (102)

When general method 2 for the synthesis of amino-esters was employed, the title compound was prepared from n-octylamine and methyl acrylate in 56% yield. The reaction mixture was heated under reflux for 9 hr. The title product had bp 110 - 112° (1 mm).

Ir spectrum (film): 3340 (N-H); 1742 (C = O) cm^{-1}.

Anal. Found: C, 66.68; H, 11.47. $C_{12}H_{25}NO_2$ requires: C, 66.93; H, 11.71.

The hydrochloride of the title compound had mp 177°.

Methyl 2-methyl-3-n-octylaminopropionate (103)

The title compound was prepared in 52% yield from n-octylamine and methyl acrylate using general method 2 for the synthesis of amino-esters and heating under reflux for 1 day. The desired product had bp 118 - 119° (0.6 mm).

Ir spectrum (film): 3350 (N-H); 1743 (C = O) cm^{-1}.

The hydrochloride was recrystallized from acetone-ether; mp 111.5 - 112°.

Anal. Found: C, 57.33; H, 10.26. $C_{13}H_{28}ClNO_2$ requires: C, 57.22; H, 10.41.

Methyl 3-(1,1,3,3-tetramethylbutylamino)propionate (104)

Using general method 1 for the synthesis of amino-esters,

the title compound was obtained from 1,1,3,3-tetramethylbutylamine and methyl acrylate in 57% yield. The reaction mixture was kept at room temperature for 7 weeks. The title compound had bp 88 - 91° (0.1 mm).

Ir spectrum (film): 3332 (N-H); 1745 (C = O) cm^{-1}.

The hydrochloride salt was recrystallized from acetone followed by a further recrystallization from methanol-ether; mp 135°.

Nmr spectrum (D_2O): δ 3.80 (3-proton singlet, OCH_3); 2.33 - 3.35 (4-proton complex multiplet $C\underline{H}_2C\underline{H}_2$); 1.70 (2-proton singlet, $CC\underline{H}_2C(CH_3)_2$); 1.47 (6-proton singlet $C(CH_3)_2$); 1.05 ppm (9-proton singlet, $(CH_3)_3C$).

Anal. Found: C, 57.50; H, 10.50. $C_{12}H_{26}ClNO_2$ requires: C, 57.22; H, 10.41.

Methyl 2-methyl-3-(1,1,3,3-tetramethylbutylamino)propionate (105)

When general method 1 for the synthesis of amino-esters was used, the title compound was prepared in 33% yield from 1,1,3,3-tetramethylbutylamine and methyl methacrylate. The reactants were kept at room temperature for 7 weeks. The compound had bp 89° (0.07 mm). Reported (Luskin et al 1956) bp 75° (0.5 mm).

Ir spectrum (film): 3340 (N-H); 1746 (C = O) cm^{-1}.

The hydrochloride was crystallized from acetone and then recrystallized from methanol-ether; mp 148 - 149°.

Nmr spectrum (D_2O): δ 3.76 (3-proton singlet, OCH_3); 2.68 - 3.61 (3-proton complex multiplet, CH_2CH); 1.70 (2-proton singlet, $C\underline{H}_2$-$C(CH_3)_2$); 1.46 (6-proton singlet, $C(CH_3)_2$); 1.26 (3-proton doublet, J = 7 Hz, CH_3); 1.04 ppm (9-proton singlet, $(CH_3)_3C$).

Anal. Found: C, 58.62; H, 10.33. $C_{13}H_{28}ClNO_2$ requires: C, 58.74; H, 10.62.

Methyl 3-allylaminopropionate (106)

When general method 1 for the preparation of amino-esters was used, the title compound was obtained in 32% yield from allylamine and methyl acrylate. The reaction mixture was kept for 7 weeks at room temperature. The compound had bp 93 - 95° (25.5 mm).

Ir spectrum (film): 3343 (N-H); 1743 (C = O); 1646 (C = C) cm^{-1}.

The hydrochloride of the title compound was crystallized from acetone and recrystallized from acetone-ether; mp 75 - 77°.

Nmr spectrum (D_2O): δ 5.28 - 6.26 (3-proton complex multiplet, CH_2 = CH); 3.60 - 3.88 (5-proton complex multiplet, $C\underline{H}_2N^+H_2CH_2CHCOOC\underline{H}_3$); 2.70 - 3.48 ppm (4-proton complex multiplet, CH_2CH_2).

Anal. Found: C, 46.85; H, 7.75. $C_7H_{14}ClNO_2$ requires: C, 46.81; H, 7.85.

Methyl 2-methyl-3-allylaminopropionate (107)

The title compound was prepared from allylamine and methyl methacrylate in 70% yield using general method 1 for the synthesis of amino-esters. The reactants were allowed to stand at room temperature for 7 weeks. The title product had bp 95° (24.5 mm). Reported (Sharifkanov and Ibranov 1963) bp 72 - 74° (3 mm); (Casy et al 1968) bp 74° (0.65 mm).

Ir spectrum (film): 3354 (N-H); 1741 (C = O); 1650 (C = C) cm^{-1}.

The hydrochloride was recrystallized (methanol-ether) and recrystallized twice more (acetone); mp 108°. Reported (Sharifkanov and Ibranov 1963) mp 103 - 104.5°.

Nmr spectrum (D_2O): δ 5.28 - 6.57 (3-proton complex multiplet, CH_2 = CH); 3.61 - 3.93 (5-proton complex multiplet, $C\underline{H}_2N^+CH_2CHCOOC\underline{H}_3$); 2.75 - 3.50 (3-proton complex multiplet, CH_2CH); 1.31 ppm (3-proton doublet; J = 7 Hz, CH_3).

Anal. Found: C, 49.69; H, 8.58. $C_8H_{16}ClNO_2$ requires: C, 49.60; H, 8.88.

Preparation of methyl 3-piperidinopropionate and its quaternary salt (108)

Piperidine (42.5g; 0.5 mol), ethyl acrylate (50.1g; 0.5 mol) and anhydrous methanol (100 ml) were heated at reflux temperature for 24 hr. after which the methanol was removed by rotary evaporation. The residue was distilled under reduced pressure to give a fraction bp 101 - 102° (13.5 mm) (72.6g). The product was re-distilled and characterized as methyl 3-piperidinopropionate (117) bp 98° (12.5 mm). Reported (Matkovics et al 1961) bp 72° (2 mm).

Ir spectrum (film): 1744 (C = O) cm^{-1}.

Anal. Found: C, 63.26; H, 10.07. $C_9H_{17}NO_2$ requires: C, 63.14; H, 10.00.

The ester base (4.3g; 0.025 mol) and methyl iodide (excess; 4g) were mixed together. A vigorous reaction set in and the product was cooled in ice-salt. After a few minutes 10 ml of anhydrous methanol were added and the methiodide precipitated by adding anhydrous ether; (4.8g; 61.3%). Recrystallization from methanol-ether gave

colorless needles, mp 147 - 148°. Reported (Matkovics et al 1961) mp 147 - 148°.

Ir spectrum (Nujol): 1741 (C = O) cm^{-1}.

Anal. Found: C, 37.94; H, 6.55; N, 4.53. $C_{10}H_{20}INO_2$ requires: C, 38.16; H, 6.87; N, 4.45.

Attempted preparation of 1,4-bis(2-methoxycarbonylethyl) ethylenediamine (109)

Ethylenediamine (0.5 mol; 30.05g) was dissolved in anhydrous methanol (200 ml) and to the solution, cooled in an ice-salt mixture, methyl acrylate (1.0 mol; 86.09g) was added portionwise. The resulting solution was kept for 3 weeks at room temperature in darkness. The solvent was then removed by rotary evaporation to leave a viscous yellow oil which was not distilled.

Attempted preparation of methyl 2-methyl-3-piperidinopropionate methiodide (110)

The free base (2.3g; 0.0125 mol) (this chemical was kindly supplied by Dr. K.K. Midha, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta who had prepared it by general method 2 for the synthesis of amino-esters) was dissolved in anhydrous methanol (100 ml), the solution cooled in ice-salt and methyl iodide (excess; 4g) added in small aliquots. The reactants were refluxed for a short time, the solution cooled, and anhydrous ether added. No title product could be isolated and attempts to prepare it using longer reaction times and different solvents such as acetone also failed. It was therefore concluded that this reaction was in some way hindered and

further efforts to isolate the quaternary methiodide were abandoned.

Methyl 2-methyl-3-dimethylaminopropionate methiodide (111)

A sample of methyl 2-methyl-3-dimethylaminopropionate, prepared by the method of Perrine (1953), was kindly supplied by Dr. K.K. Midha. The free base (3.26g; 0.025 mol) was cooled in ice and methyl iodide (excess; 4g) added dropwise. After a short time 10 ml of anhydrous methanol was added and the methiodide product precipitated by adding anhydrous ether (2.6g; 36%). It was reprecipitated from anhydrous methanol and finally recrystallized from methanol-ether giving colorless needles, mp 107 - 108.5°.

Ir spectrum (Nujol): 1733 (C = O) cm^{-1}.

Anal. Found: C, 33.57; H, 6.29; N, 5.23. $C_8H_{18}INO_2$ requires: C, 33.32; H, 6.29; N, 4.86.

2-Methyl-3-piperidinopropionamide (112)

A mixture of piperidine (21.5g; 0.25 mol) and methacrylamide (21.5g; 0.25 mol) in anhydrous methanol (50 ml) was refluxed for 40 hr. Removal of the methanol solvent by rotary evaporation left an oily residue which solidified to a yellow solid on standing. The latter was distilled using an air condenser and the fraction bp 164 - 169° (14.5 mm) collected as the crude molten title amide, solidifying on standing (24.39g; 73%). The product was redistilled and the fraction bp 178° (17.5 mm) collected. The title compound was a colorless solid, mp 90° dec.

Ir spectrum (KBr): 3374, 3196 (amide N-H); 1661 (Amide I); 1632 (Amide II) cm^{-1}.

Nmr spectrum (CCl_4): δ 7.8 and 6.79 (1-proton broad singlets exchanged in D_2O, NH_2); 2.05 - 2.82 (7-proton complex multiplet, $(CH_2)_2NCH_2CH$), 1.22 - 1.85 (6-proton complex multiplet $(CH_2)_3$); 1.1 ppm (3-proton doublet, J = 5.5 Hz, CH_3).

Anal. Found: C, 63.45; H, 9.20. $C_9H_{18}N_2O$ requires: C, 63.24; H, 9.41.

The amide base (17g; 0.1 mol) was dissolved in acetone (100 ml), the solution cooled in ice-salt and methyl iodide (excess; 20g) added in small aliquots. The reactants were refluxed for a short time to complete the reaction, the solution cooled and the product precipitated by adding anhydrous ether; (28.7g; 92%). Recrystallization from methanol-acetone afforded the methiodide as colorless crystals; mp 200.5°.

Ir spectrum (KBr): 3323; 3163 (N-H); 1689 (Amide I); 1672 (Amide II) cm^{-1}.

Anal. Found: C, 38.16; H, 6.97; N, 8.64. $C_{10}H_{21}IN_2O$ requires: C, 38.45; H, 6.78; N, 8.98.

SYNTHESIS OF AMINOHYDROXAMIC ACID HYDROCHLORIDES

General Preparative Method (Coutts et al 1969; 1970)

The appropriate amino-ester (0.05 mol) was dissolved in anhydrous methanol (20 ml) and the solution cooled in an ice-salt mixture. A solution of hydroxylamine hydrochloride (0.05 mol) in anhydrous methanol (30 ml) was then added dropwise with stirring. The reactants were then allowed to stand at room temperature from 1 day to

5 weeks. The reaction was judged complete by the absence of the strong C = O stretching band near 1740 cm^{-1} of the original amino-ester in the ir spectrum of the reaction mixture. In each case the presence of the aminohydroxamic acid was demonstrated by ferric chelate formation. When methanolic ferric chloride was added to a few drops of the reaction mixture, an intense violet color resulted. The aminohydroxamic acid hydrochlorides were then isolated and purified by the various methods indicated.

The ir spectra of the aminohydroxamic acid hydrochlorides were recorded as KBr discs or Nujol mulls. All showed a strong absorption band due to the stretching of the carbonyl group between 1630 and 1678 cm^{-1} (Coutts et al 1969: 1971), together with N^{+}-H stretching bands in the 2380 - 2780 cm^{-1} region (Heacock and Marion 1956). The nmr spectra of some of the aminohydroxamic acid hydrochlorides were recorded in DMSO-d_6. These showed a 4-proton very broad band in the δ 8.13 - 11.12 ppm range due to the two protons of the $N^{+}H_2$ group and the two protons of the NHOH group. These protons exchanged in D_2O.

2-Methyl-3-methylaminopropionohydroxamic acid hydrochloride (113)

The title compound was prepared in 44% yield from amino-ester (81) using the general method for the synthesis of aminohydroxamic acids. The reactants were allowed to stand at room temperature for 3 weeks. Anhydrous ether and acetone were then added to the reaction solution until close to the precipitation point. On standing at 0° colorless crystals of the title compound separated. The hydrochloride was recrystallized from methanol-acetone-ether: mp 137 - 138° dec.

Ir spectrum (KBr): 1665 (C = O) cm^{-1}.

Anal. Found: C, 35.40; H, 7.92; N, 16.95. $C_5H_{13}ClN_2O_2$ requires:

C, 35.62; H, 7.77; N, 16.62.

2-Methyl-3-ethylaminopropionohydroxamic acid hydrochloride (114)

Using the general method for the synthesis of aminohydroxamic acids, the title compound was prepared from amino-ester (82) in 15% yield. The reaction mixture was kept at room temperature for 1 week. The methanol was then removed under vacuum in a rotary evaporator to give an oil which was dissolved in ethanol. Addition of anhydrous ether and acetone to the solution and storage at 0° gave the hydrochloride as colorless crystals; mp 105 - 106° dec.

Ir spectrum (KBr): 1671 (C = O) cm^{-1}.

Anal. Found: C, 39.33, H, 8.22; N, 15.30. $C_6H_{15}ClN_2O_2$ requires: C, 39.46; H, 8.28; N, 15.35.

2-Methyl-3-n-propylaminopropionohydroxamic acid hydrochloride (115)

The title compound was prepared from amino-ester (83) in 23% yield by the general method for the synthesis of aminohydroxamic acids. The reaction mixture was left standing for 1 week at room temperature and the methanol concentrated by rotary evaporation. On keeping the solution at 0° the hydrochloride separated as long colorless needles. It was recrystallized twice from methanol-ether; mp 122.5° dec. Reported (Coutts et al 1970) 125 - 127°.

Ir spectrum (KBr): 1663 (C = O) cm^{-1}.

Anal. Found: C, 43.04; H, 8.70; N, 14.31. $C_7H_{17}ClN_2O_2$ requires: C, 42.77; H, 8.72; N, 14.25.

2-Methyl-3-n-butylaminopropionohydroxamic acid hydrochloride (116)

Using the general method for the synthesis of aminohydroxamic

acids, the title compound was obtained in 55% yield from amino-ester (86). The reaction mixture was kept at room temperature for 8 days. The methanol was concentrated by evaporation under reduced pressure. Anhydrous ether was added to the concentrated solution and a white solid separated. Recrystallization from methanol-ether gave colorless crystals of the hydrochloride; mp 140° dec.

Ir spectrum (KBr): 1667 (C = O) cm^{-1}.

Anal. Found: C, 45.61; H, 8.86; N, 13.38. $C_8H_{19}ClN_2O_4$ requires: C, 45.61; H, 9.09; N, 13.30.

3-tert-Butylaminopropionohydroxamic acid hydrochloride (117)

The title compound was prepared in 30% yield from amino-ester (89) by the general method for the synthesis of aminohydroxamic acids. The reactants were left at room temperature for 2 weeks. Evaporation of the solvent to dryness left an oily residue which deposited a white solid. Recrystallization from methanol-ether afforded colorless crystals of the hydrochloride; mp 168 - 169° dec.

Ir spectrum (KBr): 1678 (C = O) cm^{-1}.

Nmr spectrum (DMSO-d_6): δ 8.59 - 10.20 (4-proton very broad signal exchanged in D_2O, $N^+\underline{H}_2CH_2CH_2CONH\underline{O}H$) (Coutts et al 1971); 1.35 ppm (9-proton singlet, $(CH_3)_3C$).

Anal. Found: C, 42.90; H, 8.75; N, 13.99. $C_7H_{17}ClN_2O_2$ C, 42.74; H, 8.71; N, 14.24.

2-Methyl-3-tert-butylaminopropionohydroxamic acid hydrochloride (118)

The title compound was obtained in 66% yield from amino-ester (90) using the general method for the synthesis of aminohydroxamic

acids. The reaction mixture was allowed to stand for 2 weeks at room temperature. After concentration of the methanol on a rotary evaporator the product immediately separated out as colorless crystals of the hydrochloride; mp 181° dec.

Ir spectrum (KBr): 1677 (C = O) cm^{-1}.

Anal. Found: C, 45.53; H, 8.99; N, 13.49. $C_8H_{19}ClN_2O_2$ requires: C, 45.61; H, 9.09; N, 13.30.

2-Methyl-3-n-pentylaminopropionohydroxamic acid hydrochloride (119)

Using the general method for the synthesis of aminohydroxamic acids, the title compound was prepared in 68% yield from amino-ester (92). The reactants were allowed to stand for 10 days at room temperature. When the methanol was concentrated under vacuum in a rotary evaporator the hydrochloride crystallized to give colorless needles, mp 143 - 144° dec.

Ir spectrum (KBr): 1668 (C = O) cm^{-1}.

Anal. Found: C, 48.18; H, 9.42; N, 12.54. $C_9H_{21}ClN_2O_2$ requires: C, 48.11; H, 9.42; N, 12.47.

3-Cyclopentylaminopropionohydroxamic acid hydrochloride (120)

The title compound was prepared in 27% yield from amino-ester (93) using the general method for the synthesis of aminohydroxamic acids. The reactants were left at room temperature. After 6 days, long needles of the title compound had crystallized from the solution. A second crop of fine needles was obtained by adding anhydrous ether to the mother liquor. The two crops were combined and recrystallized from methanol-ether giving colorless needles of the hydrochloride; mp

179 - 180° dec.

Ir spectrum (KBr): 1633 (C = O) cm^{-1}.

Anal. Found: C, 45.83; H, 8.31; N, 13.63. $C_8H_{17}ClN_2O_2$ requires: C, 45.82; H, 8.65; N, 13.36.

2-Methyl-3-cyclopentylaminopropionohydroxamic acid hydrochloride (121)

Using the general method for the synthesis of aminohydroxamic acids, the title compound was obtained from amino-ester (94) in 28% yield. The reaction mixture was kept at room temperature for 1 week. Anhydrous ether was added to the solution until close to the precipitation point and on standing at 0° the hydrochloride separated as colorless crystals. It was recrystallized from methanol-ether; mp 156 - 157° dec.

Ir spectrum (Nujol): 1674 (C = O) cm^{-1}.

Anal. Found: C, 48.34; H, 8.68; N, 12.84. $C_9H_{19}ClN_2O_2$ requires: C, 48.28; H, 9.01; N, 12.53.

2-Methyl-3-n-hexylaminopropionohydroxamic acid hydrochloride (122)

The title compound was prepared from amino-ester (97) in 63% yield by the general method for the synthesis of amino-hydroxamic acids. The reactants were allowed to stand at room temperature for 18 days. The solvent was removed under vacuum in a rotary evaporator and the residual oil dissolved in anhydrous methanol. Addition of anhydrous ether to the solution and storage at 0° yielded the crystalline hydrochloride as colorless needles, mp 135 - 136° dec.

Ir spectrum (KBr): 1669 (C = O) cm^{-1}.

Anal. Found: C, 50.42; H, 9.78; N, 11.74. $C_{10}H_{23}ClN_2O_2$

requires: C, 50.30; H, 9.71; N, 11.74.

3-Cyclohexylaminopropionohydroxamic acid hydrochloride (123)

Using the general method for the synthesis of aminohydroxamic acids, the title compound was prepared in 48% yield from amino-ester (98). After being kept for 5 days at room temperature, the reaction solution deposited a white solid. The title compound was recrystallized from methanol-ether giving colorless needles of the hydrochloride. It was recrystallized several times (methanol-ether) to give material of analytical purity; mp 182 - 183° dec.

Ir spectrum (KBr): 1635 (C = O) cm^{-1}.

Nmr spectrum (DMSO-d_6): δ 8.53 - 9.02 (4-proton very broad signal exchanged in D_2O, $N^{+}\underline{H}_2CH_2CH_2CONH\underline{OH}$); 0.88 - 2.35 ppm (11-proton complex multiplet $(CH_2)_5CH$).

Anal. Found: C, 48.16; H, 8.94; N, 11.90. $C_9H_{19}ClN_2O_2$ requires: C, 48.28; H, 9.01; N, 11.96.

2-Methyl-3-cyclohexylaminopropionohydroxamic acid hydrochloride (124)

The title compound was obtained from amino-ester (99) in 67% yield by the general method for the synthesis of amino-hydroxamic acids. The reaction mixture was allowed to stand at room temperature for 16 days. Evaporation of the solvent under vacuum left an oil which was dissolved in anhydrous methanol. Dilution of the solution with anhydrous ether until close to the precipitation point followed by storage at 0° yielded the hydrochloride as colorless needles. Further recrystallizations from methanol-ether gave material of analytical purity; mp 147 - 148° dec.

Ir spectrum (Nujol): 1672 (C = O) cm^{-1}.

Anal. Found: C, 50.74; H, 9.59; N, 11.94. $C_{10}H_{21}ClN_2O_2$ requires: C, 50.52; H, 9.33; N, 11.79.

2-Methyl-3-n-heptylaminopropionohydroxamic acid hydrochloride (125)

The title compound was prepared in 66% yield from amino-ester (101) using the general method for the synthesis of aminohydroxamic acids. The reactants were kept for 10 days at room temperature. After concentration of the solution by rotary evaporation the hydrochloride crystallized as colorless needles, mp 123° dec.

Ir spectrum (KBr): 1670 (C = O) cm^{-1}.

Anal. Found: C, 51.97; H, 9.71; N, 11.19. $C_{11}H_{25}ClN_2O_2$ requires: C, 52.25; H, 9.97; N, 11.09.

3-n-Octylaminopropionohydroxamic acid hydrochloride (126)

Using the general method for the synthesis of amino-hydroxamic acids, the title compound was prepared from amino-ester (102) in 57% yield. The reaction mixture was allowed to stand for 1 month at room temperature. The solvent was removed under vacuum and the semi-solid obtained dissolved in the minimum of absolute methanol. Dilution of the solution with acetone followed by storage at 0° yielded the crude title product as a waxy solid. Crystallization from methanol and recrystallization from methanol-ether gave the pure hydrochloride as a colorless solid; mp 104° dec.

Ir spectrum (KBr): 1669 (C = O) cm^{-1}.

Anal. Found: C, 52.38; H, 10.02; N, 11.05. $C_{11}H_{25}ClN_2O_2$ requires: C, 52.25; H, 9.97; N, 11.09.

Attempted preparation of 2-methyl-3-n-octylaminopropionohydroxamic acid hydrochloride (127)

An attempt was made to prepare the title compound from amino-ester (103) by the general method for the synthesis of aminohydroxamic acids. The reaction mixture was allowed to stand at room temperature for 1 week. The solvent was removed under vacuum in a rotary evaporator and the residual oil dissolved in anhydrous methanol. Dilution of the solution with anhydrous ether until close to the precipitation point followed by storage at 0° gave a crumbly white solid. Further efforts to purify this crude material by recrystallization were unsuccessful.

Ir (Nujol): 1663 (C = O) cm^{-1}.

3-(1,1,3,3-Tetramethylbutylamino)propionohydroxamic acid hydrochloride (128)

The title compound was obtained in 68% yield from amino-ester (104) using the general method for the preparation of amino-hydroxamic acids. The reaction mixture was kept for 1 month at room temperature. Vacuum evaporation of the solvent left an oil which was dissolved in anhydrous methanol. Addition of anhydrous ether and storage at 0° afforded colorless crystals of the hydrochloride. It was recrystallized from methanol-ether; mp 153° dec.

Ir spectrum (KBr): 1630 (C = O) cm^{-1}.

Nmr spectrum (DMSO-d_6): δ 8.30 - 10.20 (4-proton very broad signal exchanged in D_2O, $N^{+}\underline{H}_2CH_2CH_2CONH\underline{OH}$); 1.71 (2-proton singlet, $C(C\underline{H}_2)\ C(CH_3)_2$); 1.40 (6-proton singlet, $C(CH_3)_2$); 1.01 ppm (9-proton singlet, $(CH_3)_3C$).

Anal. Found: C, 52.39; H, 9.75; N, 11.03. $C_{11}H_{25}ClN_2O_2$ requires: C, 52.25; H, 9.97; N, 11.09.

2-Methyl-3-(1,1,3,3-tetramethylbutylamino)propionohydroxamic acid hydrochloride (129)

Using the general method for the preparation of amino-hydroxamic acids, the title compound was obtained in 44% yield from amino-ester (105). The reactants were allowed to stand at room temperature for 5 weeks. The methanol was removed under vacuum in a rotary evaporator to give a colorless solid. Recrystallization from methanol-ether gave colorless crystals of the hydrochloride; mp 172° dec.

Ir spectrum (KBr): 1673 (C = O) cm^{-1}.

Nmr spectrum (DMSO-d_6): δ 8.60 - 10.93 (4-proton very broad signal exchanged in D_2O, $N^+\underline{H}_2CH_2CHCONH\underline{OH}$); 1.73 (2-proton singlet, $CC\underline{H}_2C(CH_3)_2$)), 1.42 (6-proton singlet, $C(CH_3)_2$); 1.18 (3-proton doublet, J = 7 Hz, CH_3); 1.01 ppm (9-proton singlet, $(CH_3)_3C$).

Anal. Found: C, 54.08; H, 9.99; N, 10.72. $C_{12}H_{27}ClN_2O_2$ requires: C, 54.01; H, 10.20; N, 10.50.

Attempted preparation of 3-allylaminopropionohydroxamic acid hydrochloride (130)

The synthesis of the title compound from amino-ester (106) was attempted using the general method for the preparation of amino-hydroxamic acids. The reactants were kept at room temperature for 6 days. Evaporation of the solvent to dryness left an oily residue which could not be crystallized.

2-Methyl-3-allylaminopropionohydroxamic acid hydrochloride (131)

The title compound was prepared from amino-ester (107) in

31% yield using the general method for the synthesis of aminohydroxamic acids. The reaction mixture was allowed to stand at room temperature for 3 weeks. Evaporation of the solvent left an oily residue which was dissolved in absolute methanol and diluted with acetone. Storage at 0° afforded the hydrochloride as colorless needles, mp 60° dec.

Ir spectrum (KBr): 1664 (C = O) cm^{-1}.

Nmr spectrum (DMSO-d_6): δ 8.13 - 11.12 (4-proton very broad peak exchanged in D_2O, $N^{+}\underline{H}_2CH_2CHCON\underline{H}O\underline{H}$); 5.24 - 6.39 (3-proton complex multiplet CH_2 = CH); 3.46 (2-proton doublet, J = 5.5 Hz, $CHC\underline{H}_2$); 1.16 ppm (3-proton doublet, J = 6 Hz, CH_3).

Anal. Found: C, 43.36; H, 7.54; N, 14.49. $C_7H_{15}ClN_2O_2$ requires: C, 43.21; H, 7.77; N, 14.40.

CHAPTER 7

EXPERIMENTAL PHARMACOLOGY

BIOLOGICAL METHODS

ANAESTHETIZED RAT EXPERIMENTS

All experiments were performed on rats weighing between 160 and 530g. The rats were anaesthetized with urethane (0.75 ml per 100g intraperitoneally of a 25% solution). The surgical procedures were similar to those given by D'Amour et al (1965). The animal was fastened by each limb to a dissecting board with pieces of adhesive tape. An incision was made in the mid-line from slightly in behind the chin to the upper part of the thorax. The overlying connective tissue was cut and the underlying muscles separated along their central line to expose the trachea. The trachea was separated from the oesophagus by breaking the connective tissue holding them together. An opening was cut in the trachea and a tracheal cannula (a short length of Intramedic PE 240 polyethylene tubing with a tapered end) tied in place.

The arterial cannula (a length of Intramedic PE 50 polyethylene tubing with the free end tapered) was connected to an E & M Instrument Co. Inc. Transducer, Model P-1000-A, via a size 23G 1 needle. The system was filled completely by flushing an anticoagulant saline solution (Heparin, 1000 units per ml) through the transducer. The cannula itself was then filled separately with Heparin solution (10,000 units per ml). The transducer was then connected to an amplifier and pen recorder (E & M Physiograph Desk Model DMP-4A) and calibrated.

One of the carotid arteries was exposed and separated from its connective tissue sheath and from the vagus and cervical

sympathetic nerves for a distance of 1 - 2 cm. The artery was tied off as far anteriorly as possible and the posterior end clamped with a bulldog clip. A small snip was made as close to the anterior tie as possible and the carotid cannula inserted into the artery and tied off in front of the bulldog clip. The clip was removed, the circuit opened to the transducer and the arterial blood pressure recorded.

Two techniques were employed for the introduction of drugs into the system. With larger animals it was found convenient to cannulate the femoral vein. The femoral artery, vein and nerves were exposed and the vein separated from the artery. Two ligatures were positioned under the isolated vein, a small snip made in the vein and the posterior ligature tied off. The venous cannula (a length of Intramedic PE 50 polyethylene tubing partly drawn out, with the free end tapered) was attached to a 1 ml syringe fitted with a blunted size 23G 1 needle. The cannula was filled with saline from the syringe, inserted into the vein, and gently pushed down the vessel to a distance of about 2 cm. The anterior ligature was tied and an additional ligature sewn through the leg tied down on the cannula to prevent it slipping out during handling.

An alternative technique, found somewhat more convenient with smaller sized animals, involved cannulation of the jugular vein. The vein was carefully dissected free and the anterior end tied off. A small snip was made in the vein and the jugular cannula inserted, (facing the heart) and tied in position. As before an additional ligature was tied down on the cannula tubing to prevent its slipping out.

The drugs were dissolved in saline immediately before use and introduced via the venous cannulae using 1 ml disposable syringes attached to blunted needles. After injection each drug was washed into the system with 0.2 ml of saline.

ISOLATED RABBIT INTESTINE EXPERIMENTS

The rabbit intestine preparation described by Finkleman (1930) was used. A rabbit was killed and a length of ileum was taken with the mesenteric attachments intact. Portions 2 - 3 cm in length were cut to which the mesenteric blood and nerve supply could be clearly seen. Threads were attached to this and to each end of the piece of intestine and the tissue suspended in a jacketed 20 ml or 300 ml organ bath. The mesentery was then threaded through electrodes connected to a stimulator (Harvard Apparatus Co. Model 240). A modified Krebs solution was used of the following composition in g per 1 of distilled water: NaCl, 6.9; KCl, 0.35; $CaCl_2 . 6H_2O$, 0.36; $MgSO_4 . 7H_2O$, 0.29; $NaHCO_3$, 2.1; KH_2PO_4, 0.16; glucose, 1.0. The solution was equilibriated with 95% oxygen and 5% carbon dioxide and the temperature was maintained at 37°. The spontaneous contractions of the muscle were recorded by means of a strain gauge and an E & M Instrument Co. Inc. Isotonic Myograph Transducer, Mk II Ser 670, connected to an amplifier and pen recorder (E & M Physiograph Desk Model DMP-4A).

EXPERIMENTS TO DETERMINE THE EFFECTIVENESS OF AMINOHYDROXAMIC ACIDS AGAINST DFP POISONING IN MICE

Male Alas strain mice of weight range 20 - 25g were used.

Great care was exercised in handling DFP. It was transferred to a weighed sample vial in a fume hood, dissolved in distilled water and diluted such that 0.1 ml contained 5 mg/kg when administered ip. Each 3-monoalkylaminopropionohydroxamic acid hydrochloride was made up as a solution in distilled water so that 0.2 or 0.3 ml contained the desired dose on sc injection. Alternate routes were used for the injections of the aminohydroxamic acids and the DFP to minimize direct interaction between the two compounds.

The general procedure was to inject groups of mice with the desired dose of the compound under investigation followed either 20 or 30 minutes later by an injection of DFP. In each set of experiments one group of mice in which distilled water instead of an aminohydroxamic acid had been injected was used as a control.

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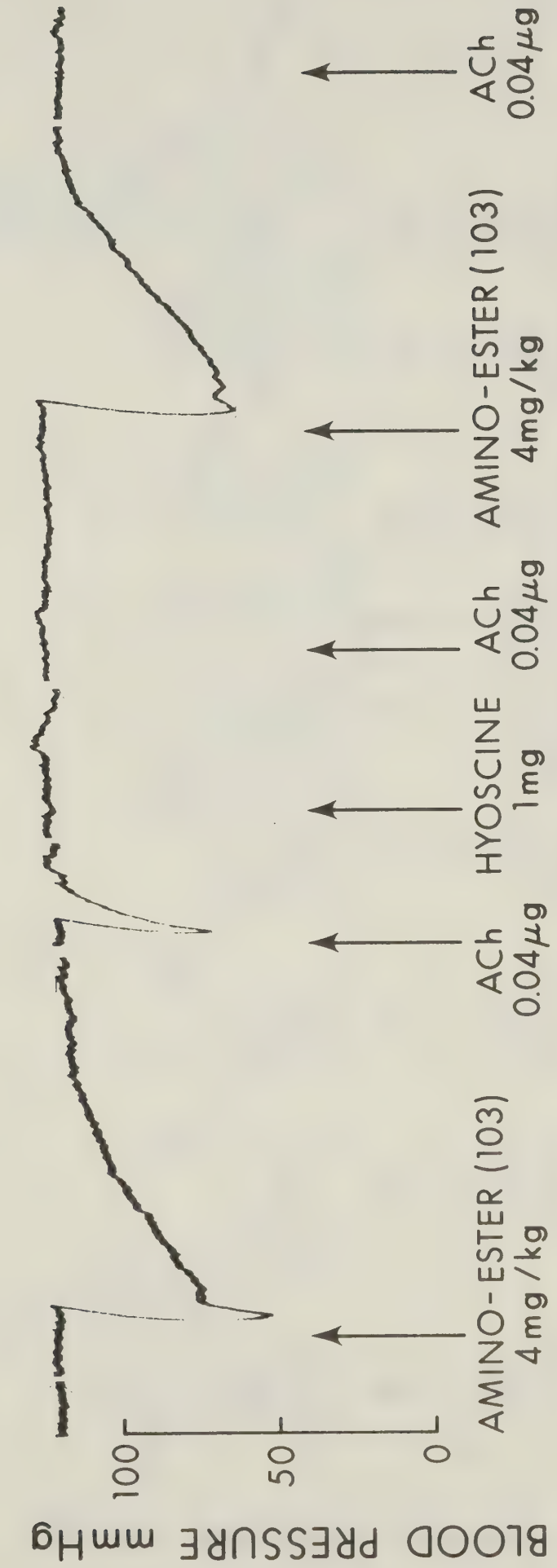

Figure 1. Effects of 2-methyl-3-octylaminopropionate hydrochloride (103) on the blood pressure in hyoscine-treated rats. Note that ACh (0.04 µg) and 103 (4 mg/kg) produced a fall in the blood pressure before treatment with hyoscine. Note also that after hyoscine, the response to ACh (0.04 µg) was completely blocked, while the response to 103 still persisted.

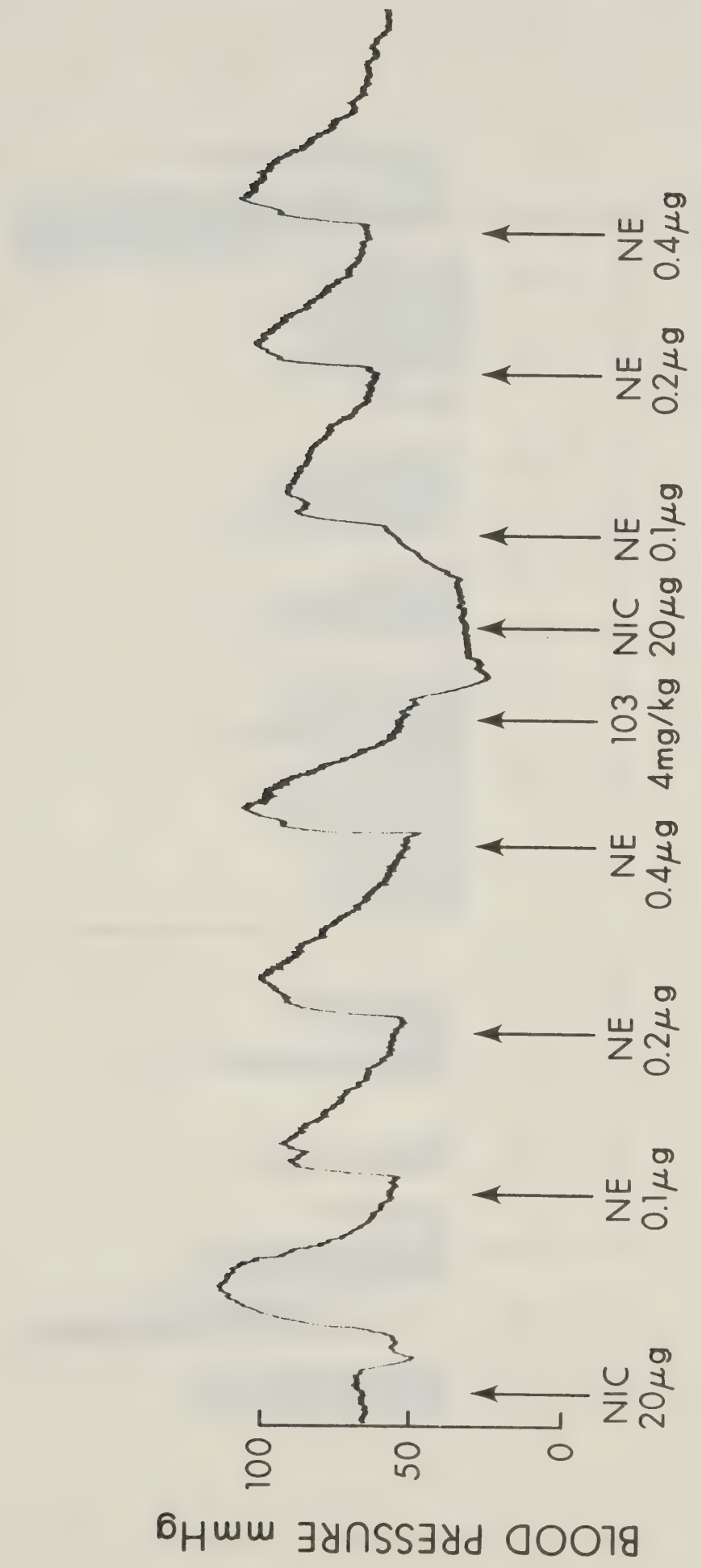

Figure 2. Effects of nicotine (NIC) and norepinephrine (NE) on the blood pressure of rats treated with 2-methyl-3-octylaminopropionate hydrochloride (103). Note that the pressor response to nicotine was completely blocked in the presence of 103, while the pressor response to norepinephrine was only slightly lowered.

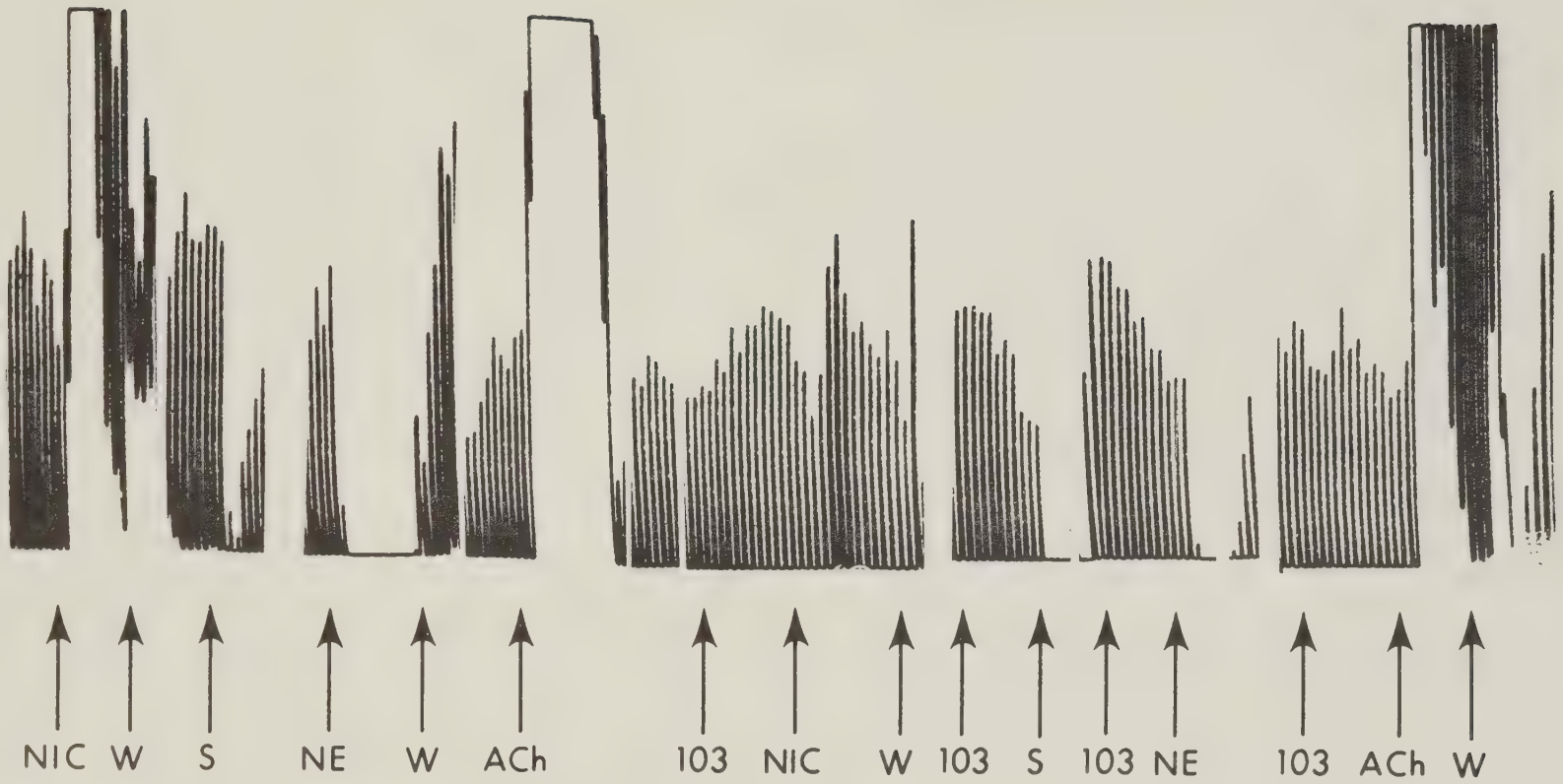

Figure 3. The effects of 2-methyl-3-octylaminopropionate hydrochloride (103) (2.5 µg/ml) on the responses of the isolated rabbit intestine to nicotine (NIC) (1 µg/ml), sympathetic nerve stimulation (S) (150 volts at a frequency of 10/sec), norepinephrine (NE) (0.1 µg/ml) and ACh (0.05 µg/ml). The arrows indicate the start of exposure of the tissue to the drugs, stimulation of the sympathetic nerves or washings with modified Krebs solution. Note that 103 completely blocked the effects of nicotine, while the responses to sympathetic nerve stimulation, norepinephrine and ACh could still be obtained.